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Taking stock of *Nature's* bread and butter

VERY occasionally we get our leg pulled about the words on our refereeing form. Our standards call for "topicality, brevity and plausibility". "You'll find this paper seductively plausible", purred an author in a letter of transmittal. "Plausible—yes; correct—no", responded a referee. So we went back in some diffidence to Fowler, who generally gets it right—

'**plausible** has moved a long way from its original meaning, "deserving of applause". Applied to a person it is always pejorative; a plausible man is one who obtains a credence he does not deserve. Applied to an argument the word has not travelled so far on the downward path: it may still be used of one that commends itself, though speculative.'

Continue to send us plausible papers. We will do our best not to send them to plausible referees.

- It is no doubt foolish to boast of the turn-round time for manuscripts, but since it forms the basis on which many scientists decide where to publish, here goes. Once a manuscript has been accepted, it will, on average be published within six weeks.

Statistics on the refereeing process are far more variable, of course, as it may take anything from a week upwards to satisfy ourselves and a referee that a paper should or should not be published. Not all papers go to referees. Some of those submitted seem to us more obviously suited to a specialised journal; and after taking appropriate opinions around the office—and often outside—we return such manuscripts as soon as possible. There is little point in seeking a referee's opinion and only then over-ruling it with our own opinion.

Of the remaining manuscripts, we seek a referee's views on all but a tiny fraction. The tiny fraction are not, as is widely believed, those written by very eminent men, but papers on matters where the facts are indisputable and delay would be positively detrimental to the community.

Some authors feel that sending a paper to a distinguished scientist for him to transmit does some good. It doesn't—it only profits the Post Office.

- One of the greatest problems encountered by sub-editors is that of ambiguity. This is exceptionally acute in a journal in which more than half the readers and many of the authors do not have English as their first language. English is undoubtedly an organic language capable of great flexibility and subtlety through the ease with which it handles nouns as adjectives, absorbs new words, possesses many words meaning almost the same thing and so on. Asset though this is, particularly in the spoken language, it does lead to difficulties for the reader—and not only the reader to whom English is a second language.

It is impossible to set down guidelines; ambiguities follow no rulebook. But here are some which may

give some idea of the subtleties which readers are sometimes asked to unravel.

Jones is a solid state physicist (or soft rock geologist).

The equipment has provided extensive trouble free operation.

Smith's paper suggests a new concept (to Smith or to the author?)

Since Robinson made the observations, we have confidence in the theory.

Brown's work appeared to resolve the difficulty.

We shall hopefully repeat this work within a month.

The solution boiled momentarily.

(These last two demonstrate that the generation of ambiguous euphemisms for 'I hope' and 'in a moment' has effectively eliminated two words from the language.)

Some of these examples seem trifling to the English speaker; but the test we try to apply (not always with success) is whether the writing would be clear not only to someone not having English as a first language, but also to someone from a remote discipline reading out of curiosity.

- It is always tricky to know what references are really necessary; to the extent that we attempt at one and the same time to carry large numbers of short scientific reports and also to have them widely accessible we cannot avoid some conflict over the amount of referencing. But on the whole, we prefer relatively few references because anyone who finds interest in a paper outside his own field is presumably going to look and talk further. Particularly vulnerable to the sub-editor's pen are sentences that declare: "There has recently been great interest in this subject¹⁻¹¹ and much work¹²⁻²⁵ is at present in progress".

- Many people secretly fancy themselves as advertising copywriters and would relish the opportunity to string together compelling but honest words to persuade others to buy cars, read books or (dare we say) to drink a certain brew or smoke a certain cigarette. And yet given an opportunity for self-advertisement, most scientists shy away from compelling but honest words with which to introduce their papers.

Nature has always avoided a summary at the beginning of letters partly for reasons of space, partly in an attempt to retain a certain directness in the reporting of early and often tentative results. But this should not inhibit anyone from writing a short and forthright first paragraph, capable of being understood by almost anyone. On occasions our sub-editors attempt to do this themselves for authors, but the response is often: "Well I didn't quite mean that". Which might, just once in a while, not stem entirely from our dim-wittedness but conceivably from a touch of opacity in the paper itself. □



Updating the Green Revolution

BY DAVID SPURGEON

PERHAPS the most important problem facing agricultural scientists in the decades ahead is how to keep production of staple foods like rice abreast of population increase in the Third World. In Asia alone, something like 5 million tons of rice will have to be produced every year to keep up with the demand. That means that new varieties and agronomic practices must spread, and that new lands must come into production. It also means that pests and flooding must be controlled or their effects minimised and that, in an era of inflation and rising prices, the costs of technological inputs such as fertilisers must be reduced.

One of the chief reasons that the success of the Green Revolution has been limited so far is that the total area in which the new seed varieties and agronomic practices were applied has been small. In the case of rice, the high yielding varieties were semi-dwarf types suitable only for shallow water regions (depth approximately 5–50 cm). These lands constitute only 25–30% of the world's total rice lands and even in these regions, of course, the new varieties are not grown exclusively. The Green Revolution has scarcely touched the other rice-growing areas—the uplands and regions of intermediate and deep water that together comprise 65–70% of the total available rice lands.

The International Rice Research Institute (IRRI) in the Philippines, where the new varieties and technology were developed, is at present working, in collaboration with rice scientists and growers elsewhere, to extend the Green Revolution to the majority of the world's rice-growing areas—and to solve some of the other problems that limit rice production. Some of them feel that rice production could be doubled throughout the world within the next 15 years through such methods. According to Dr Gurdev Kush, head of the IRRI's plant breeding department, the situation is not that gloomy when the available resources are considered. The problem is exploitation of the resources.

The really limiting factors in food production are political, Dr Kush believes, and in addition vast areas of Africa and Latin America have not been exploited. "I'm convinced," he



says, "that if there's no major economic upheaval, and provided population control programmes are maintained, we can keep up with demand for the next 15–20 years."

One of the rice-growing sectors of the world being looked at with special interest by IRRI scientists contains the deep water regions, because these are among the most heavily populated anywhere. People have lived for centuries along the deltas of the mighty rivers of Asia—the Ganges, the Brahmaputra, the Godavari, the Irrawaddy, the Chao Phraya and the Mekong—because of the adequate supply of water and the rich silt deposits.

Often, floating rice is the only food crop that farmers can grow in these areas, but the tall varieties found there are low yielding: for decades they have produced only 1 or 2 tons a hectare. Almost 10% of the rice land in Asia and Africa is planted with floating rices, whereas almost half the total rice land has tall non-floating varieties that are adapted to medium-deep water.

Last summer, the first International Seminar on Deep Water Rice was held at the Bangladesh Rice Research Institute north of Dacca—just after flood waters had destroyed much of that country's rice crop. The disaster served as an object lesson for the group, which surveyed both the flood damage and deep water rice culture from helicopters and boats, noting fields where semi-dwarf and even tall varieties were submerged and killed in fields adjoining flourishing deep water rice.

These floating varieties have developed genetic mechanisms that allow them to grow normally in deep water and to escape flood submergence—their stems elongate (for reasons not under-

stood) as waters rise and their leaves float on the surface. Rice scientists are now incorporating this genetic characteristic into semi-dwarf and intermediate stature rices, hoping to develop high yielding varieties that can survive floods. Several thousand hectares of an IRRI strain known as IR442-2-58 are now being grown in West Bengal, India, where flood waters often reach a depth of 1 m. More than 40 other crosses have been made between floating and semi-dwarf types, and progeny are being tested in Bangladesh, India, Vietnam, Indonesia and Thailand. The headquarters for the deep water programme is at the Hunter Deep Water Rice Research Centre in Thailand.

"Not many scientists are really experts on deep water rice, despite the extent of the deep water areas and the huge populations they support," says Dr Mohammed Amirul Islam, Director of the BRRI. "This makes it all the more important that attention be focused on the urgent problems of deep water rice."

Other rice types being developed are varieties that will not elongate like floating rices, but will withstand submergence for a few days under flood conditions. These varieties would be useful where flooding is temporary: the floating varieties might lodge (topple over) as floodwaters recede. The need is illustrated by an incident in the Philippines in 1972, where about 230,000 hectares of rice land were flooded in early growth stages for four weeks, causing a crop loss of about \$74 million.

Still other varieties of rice are being developed to adapt to the opposite of flood conditions: drought. About 10–15% of the world's rice-growing areas



Rice trials field in the Far East

hieved with disease and insect resistance. Early this year the IRRI named three early maturing rice varieties (IR 28, 29 and 30) as being resistant to a number of diseases and insects. They are the only known varieties highly resistant to grassy stunt virus disease, a serious problem in many countries. The varieties IR 28 and IR 29 are also resistant to tungro, bacterial blight and blast diseases; IR 30 is resistant to tungro and bacterial blight but moderately susceptible to blast. And all three varieties are resistant to brown plant hoppers and green leaf hoppers and moderately resistant to stem borers.

Furthermore, IR 28 and IR 30 mature in only 105 days and IR 29 in 115 days, compared with about 160 days for traditional tropical varieties and 130 for most high yielding varieties. These short seasons should enable farmers in some areas to adopt multiple cropping systems, thus cutting their risks. Varieties that mature in shorter periods are less likely to be damaged by insects or disease or weather simply because they are in the field for a shorter time. In addition, some farmers might fit in an extra crop this way.

Bad soil conditions prevent farmers from growing rice on millions of hectares. Felix Ponnamperna, a Ceylonese soil chemist at the IRRI, visited Bombay and found that just 10 miles outside the city there were 100,000 hectares of land ideally suited to rice cultivation yet unusable because of salinity, whereas inside the city people were dying of malnutrition. This is flat, alluvial soil, and there is water throughout the year. There are about 10 million hectares of saline soils in India and Pakistan.

In Indonesia new lands are being opened up, says Dr Ponnamperna, but they are largely peaty bogs. The country hopes to open up five million hectares in the next 10 years, but they

contain problem soils, and rice does not grow well on them. In all there are about 40 million hectares in the tropics composed of these marsh soils, mostly in South-east Asia.

Scientists now are studying the deficiencies of these soils—deficiencies that include almost all the major nutrients and some trace elements. Even when these elements are added they have found that rice growth is not as good as it should be, probably because of the presence of organic toxins. "So we want to breed varieties adapted to these conditions," says Dr Ponnamperna. "We are trying to tailor the crop to the soil rather than the reverse."

Problems include acid sulphate soils (in perhaps 15 million hectares on which rice cannot be grown in Vietnam and Thailand); iron toxicity when certain soils are flooded (affecting 10–50 million hectares of rice soils), retarding growth and limiting yield; and zinc deficiency (affecting 500,000 hectares in the Philippines and large areas of Pakistan and India). The IRRI found in the Philippines that zinc deficiency sometimes prevented rice growth altogether, but that dipping seedling roots in zinc oxide and water was the answer. (Only \$1 worth of zinc oxide per hectare (1 kilogram) often produced higher yields than 550 kilograms of complete fertiliser). And with zinc oxide seedling dip, any of the modern varieties of rice could produce twice the yield of the local varieties, even without any other fertiliser.

A third of mankind—1,300 million people—depend on rice for more than half their food. Ninety percent of the low income people of the most densely populated regions of the world rely on it, people whose average income is only \$80. The efforts of the world's rice scientists will be crucial to their survival in the years ahead. □

suffer from insufficient water supply, for example in the uplands. Varieties tolerant to drought are being sought that will, nevertheless, recuperate quickly when the rains come, for areas of varied rainfall. Many regions of the world are too cold for the new varieties yet local rices grow there. Scientists at the IRRI are now gathering seeds from these rices and growing them in ice water to see which ones will survive and then to try to cross breed them.

The IRRI calls this programme of rice improvement the Genetic Evaluation and Utilisation Program (GEU). It brings together not only plant breeders but pathologists, entomologists, soil chemists and agronomists in order to identify and evaluate systematically rice genetically adapted to the most pressing constraints on rice production.

Besides the attributes mentioned, the programme seeks to incorporate these other factors into new high yielding varieties: resistance to insects, resistance to diseases, high protein levels and tolerance to toxic soils. In addition scientists are screening rice varieties to determine which use fertiliser and other agricultural chemicals most efficiently, and how best to deliver the chemicals.

It has been found, for example, that, if fertiliser is incorporated in a kind of mud pellet and placed near the plant's roots, about half the amount of fertiliser normally used will produce the same yield. In other words, the method doubles the efficiency of the chemicals. The difficulty is to package the fertiliser and insecticide together in a suitable way and to persuade industry to produce it.

Some success has already been ac-

Resistance ratings of IRRI varieties

Variety	Diseases				Insects			Soil problems			
	Blast	Bacterial blight	Grassy stunt	Tungro	Green leaf-hopper	Brown plant-hopper	Stem borer	Alkali injury	Salt injury	Zinc deficiency	Phosphorus deficiency
IR8	MR	S	S	S	R	S	MS	S	MR	S	MR
IR5	S	S	S	S	R	S	S	S	MR	R	MR
IR20	MR	R	S	R	R	S	MR	S	MR	R	R
IR22	S	R	S	S	S	S	S	S	S	S	MR
IR24	S	S	S	MR	R	S	S	MR	MR	S	MR
IR26	MR	R	MS	R	R	R	MR	MR	MR	S	R
IR28	R	R	R	R	R	R	MR	MR	MR	R	R
IR29	R	R	R	R	R	R	MR	S	MS	R	R
IR30	MS	R	R	R	R	R	MR	MR	MR	R	MR

R = resistant MR = moderately resistant MS = moderately susceptible S = susceptible

Energy in the OECD

G. R. Bainbridge, Professor of Energy Studies and Director of The Energy Centre, University of Newcastle upon Tyne, reflects on the OECD report, Energy Prospects to 1985.

WHEN, in 1972, the council of the OECD initiated an assessment of energy developments and related policies it was anticipating that the projected growth of energy demand in the 24 countries constituting the OECD area would put increasing pressure on low cost energy supplies. It was particularly concerned about future supplies of oil from the Middle East, where 90% of the world's petroleum trade originates and where 55% of the proven natural oil reserves lie.

Moreover the USA, using a third of the 5,000 million tons of oil equivalent world annual energy consumption, was becoming increasingly an oil importer. Indeed only two OECD countries, Canada (trading oil and gas for coal) and Australia (trading coal for oil) have net total energy self sufficiency. So the pressure of oil demand would inevitably have important implications for the economies and policies of most OECD members, 15 of whom are dependent for 70% or more of their energy needs on imports, and only some of whom (the USA, Norway, the Netherlands and UK) could with sustained effort join the select group of those reasonably self sufficient in energy.

The concerted oil export restrictions applied by OPEC towards the end of 1973, followed by a dramatic increase in crude oil prices, came as a shock, emphasising the timeliness of the OECD energy exercise. It was clear that the prospects for a return to an era of relatively cheap energy had "dwindled considerably".

It is not surprising that the OECD assessors found themselves unable to provide probable, or target, projections of energy supply and demand, even for 10 years ahead, while initiatives were and still are being taken by the oil suppliers and consumers almost daily. They made, instead, a brave attempt to present in a qualitative way the principal likely determinants of future development of the energy sector. Increasing gross national production would be expected to require more energy, though perhaps not too much more if the trend towards improved energy-efficient processes continues

and the likely substitution of capital and labour for more costly energy use occurs.

The price of energy had scarcely before been considered explicitly as a factor influencing energy demand, because prices were low and fairly stable. Now it had become the main one. In obtaining estimates of future energy use, oil prices rising from the early 1973 baseline of \$3 (USA 1972 money) a barrel (7 barrels~1 tonne) to \$6 and \$9 were taken to provide a framework for investigating the implications for other economic factors and to identify possible problems that might confront the policymaker. Without criticism they could have gone even higher with the alternatives. During the period of the exercise, of course, dollar depreciation made those prices selected \$3.60, \$7.20 and \$10.80 by the end of 1974. Inflation is only one of several factors listed in the recent Solemn Declaration of the Sovereigns and Heads of State of the OPEC member countries for consideration by them when adjusting crude oil prices in future.

By comparison with growing gross national product and primary fuel prices the other determinants of energy supply and demand tend to fade into insignificance, particularly in the next worrying decade of readjustment. Measures already taken or planned to improve environmental protection were found to tend to increase energy consumption, albeit by only a few percent. Although some of them have already been relaxed or left in abeyance to ease the energy crisis, efforts towards energy savings, if successful, could enable environmental protection measures to be resumed.

As might be expected the main part of the OECD report concentrates on the prospects for the provision of oil, coal, natural gas, electricity and nuclear power in the near future. The possibility of greater indigenous production within the OECD is considered particularly important, with nuclear fission, North Sea oil and gas, and rejuvenation of coal and its synthetic products being all seen to have useful parts to play.

In order to broaden the options for future choices, and to open up greater possibilities for the diversification of energy supplies, continuing research and development efforts directed towards exploitation of some of the renewable resources are recommended in the report. The contributions from solar energy and further geothermal energy were recognised to be expected in general beyond 1985. Research into the derivation of fuels from towns' wastes and the productions of methanol and hydrogen was also recommended, with thermonuclear fusion as the *pièce de résistance* for the long term. Little was

said of wind and wave power, or ocean thermal gradients, as an energy source, perhaps because the prevailing South-westerlies and the Gulf Stream have apparently been veering a little off course of late.

Anyone preparing to read this very informative OECD report should apply initial energy to acquiring a good magnifying glass, as the many and interesting tables in the key chapters 2 (Energy Demand and Supply Projections) and 3 (Economic and Environmental Implications) are printed minutely. Chapter 4, on Energy Conservation, is appropriately of below average length, but that glass is needed again for the vital table which analyses energy conservation possibilities; but the effectively blank four pages immediately preceding the chapter emphasise the scope there is for workers in the energy field to practise what they preach. More than 5% of the 224 pages in Volume 1 carry no significant point and the proportion is higher in Volume 2.

Because of the dominance of US energy consumption (and production) in any overall OECD energy trends, it was correct that the USA should be treated separately. In 1972 the USA was using 50% more primary energy than the 19 European OECD members together, and its indigenous production was three times greater than theirs. Separate consideration of Canada was also useful, for, although it uses only a tenth as much energy as the USA, its surpluses of oil and natural gas will increasingly be directed southwards. Japan (also separately treated) already imports more energy than the USA and in future will continue as a major competitor for the world's oil, coal and natural gas resources—unlike the USA, Japan has little scope for indigenous production and has a higher growth rate for both population and gross national product.

The EEC is included for statistical comparison in some of the tables of the report. The strength of the EEC compared with the OECD European 19 lies in the fact that the smaller grouping contains the major industrial countries, using well over 50% of the primary energy, producing over 70% of the European indigenous fuel supplies and with the better natural potential to improve on that position if pressed. The energy strength of the EEC might have been further improved by Norway, but that country chose through a referendum to opt out; it has indigenous North Sea oil and gas production potential of the same order as the UK, but a very much smaller population already well endowed with hydro-power resources.

The figure summarises historical behaviour and possible alternative trends

of future OECD energy requirements. Had world oil prices not increased, the doubling time for energy consumption would be running at about 15 years, some 4.9% a year. Although the OECD, with present energy consumption running at a little over 3,500 million tons of oil equivalent a year, accounts for about 70% of world usage, the developing countries outside the OECD group will tend to bias world growth to a higher rate and a shorter doubling time. With the oil import price increased by factors of 2 and 3, some energy savings are anticipated to bring OECD growth down to 4.3% a year and 3.8% a year respectively. Then, at 1985, the higher oil prices have depressed energy use (or encouraged desirable energy savings) to the extent of 7% and 12% respectively, on the total. The nuclear, coal and gas proportions are all increased for the higher oil prices, relative to oil.

Some of the immediate broad conclusions could have been deduced by intuition almost as easily as from detailed analysis, namely:

- The OECD share of oil is expected to decline to below 50% of total energy requirements from around 55%, though its use is still increasing steadily at 1985 and seems likely to have reached at least 2,800 million tons of oil equivalent a year, compared with 2,000 million tons of oil equivalent a year today. The increment alone is a factor of 4 higher than the anticipated oil yield from the North Sea.

- The natural gas share grows on balance, the percentage increment in Europe compensating for a relative decline in use in the USA. Although the percentage stays around the 22% mark, the annual use almost doubles from 600 to about 1,200 million tonnes of oil equivalent. It should be noted that natural gas is being increasingly supplied into the Eurogas grid from Russia and Holland; soon additional supplies will come north from Africa by undersea pipeline by way of Italy, supplementing present liquid natural gas trans-shipments.

- Nuclear power is expected to play a steadily increasing role, rising (including a small component of hydro) from around 4% of total energy now to some 8 to 10% by 1980 and 12 to 16% by 1985. As might be expected, therefore, the nuclear doubling time is of the order 5 to 7 years, below half that for total energy. This is significant in view of the long lead times for power station construction, and must depend on policy decisions already taken in many countries to press on more quickly with nuclear programmes in view of the higher oil prices.

- The use trend for coal is expected to increase again, reversing a steady decline after the Second World War. European coal resources are mainly in Germany (brown) and the UK (black) but the USA still has vast and relatively easily worked coal deposits. It is there and in Australia that the most likely prospect for greatly raising coal production arises, though in Europe an increase of 10 to 20% seems to be a sufficiently challenging and achievable target.

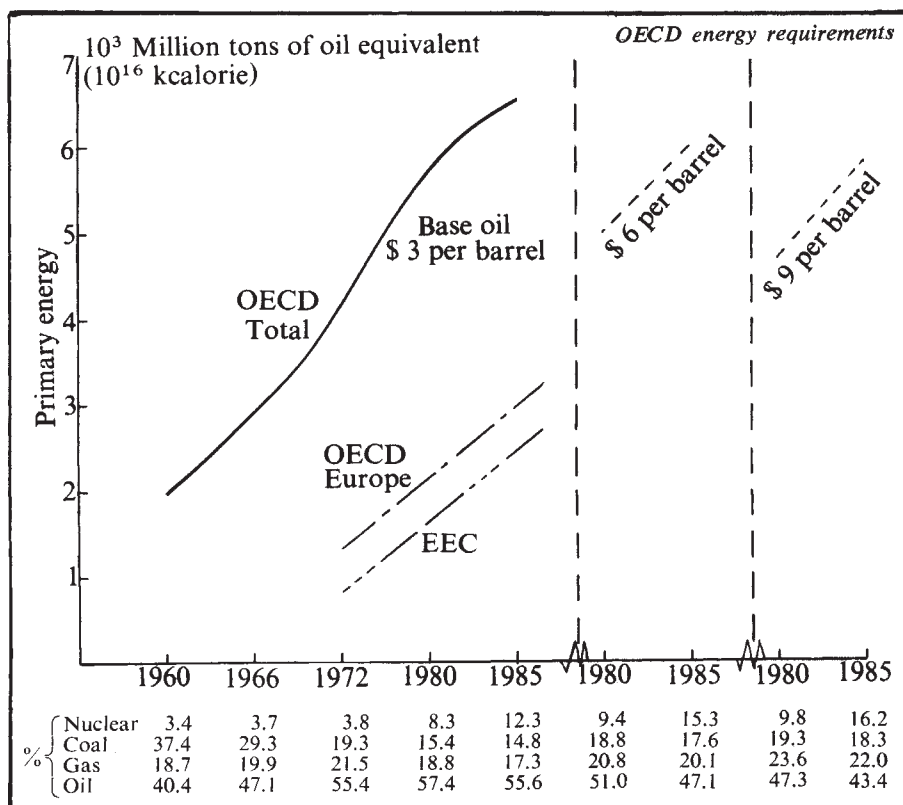
The analysis leading to the projections does not seem to have allowed for the probability that coal and natural gas prices are bound to rise to what the market will bear for these fuels, relative to the oil price, or that nuclear capital costs will escalate because of the effect of increased conventional fuel and labour costs on manufacture. These occurrences will depress the demand for fuels alternative to oil, and the supply of oil may exceed demand sufficiently for selling competition to re-enter the market. The report is right to suggest that, because the energy pattern of the OECD and indeed the world, is a dynamic one, continuing study to achieve a better understanding of it is worthwhile. For example, OECD oil imports in 1980 could easily be 70% higher than now if savings of energy use, substitution of other energy forms and restriction of national product outputs are not effected. Higher oil prices, if sustained, could, however, reduce the extra import demand to below 20% and cause even a reduction of the

amount of imported oil and expenditure on it, ignoring any oil suppliers' price adjustments to optimise their returns.

Actions recommended to OECD members to improve the arrangements for cooperation in energy and related fields, inter-regional (EEC and OECD Europe) and intra-regional (Europe-North America, Europe-Far East) if possible, seem laudable; emergency energy-sharing schemes, promotion of energy trade, energy savings schemes, energy research and development to ensure adequate supplies at reasonable cost all need work. Inflation, and the sorting out of balance of payments problems, are current international problems needing OECD support.

A bringing together of the oil-exporting and oil-importing countries in agreed policies for mutual and world benefit is another area recommended for OECD action, with joint studies of problems to find agreed solutions which will bring greater stability to the energy and money markets. The idea that scientific and technical assistance should flow from countries with the capability towards those that need to develop is one on which OECD and OPEC representatives should be coming close together.

In the UK Parliamentary debate on the EEC energy proposals early in February of this year it was concluded that as trade with our partners becomes more interdependent there is obvious sense in seeking to evolve coordinated energy policies. The OECD report firmly confirms that view. □



international news

EARLY last year, the revelation that Canada's National Research Council (NRC) had for many years served as a cover for the secret gathering of intelligence shocked many Canadians. It also led the then leader of the New Democratic Party, David Lewis, to propose shifting the NRC unit concerned (the so-called Communications Branch) into a more appropriate department, and Prime Minister Trudeau to promise that he would consider the suggestion.

A few weeks ago, the Canadian public learned that the question had indeed been considered: in fact the Communications Branch had been transferred to the Department of National Defence. The information came out in questioning of the Minister of State for Science and Technology, C. M. Drury, in the House of Commons.

But the matter didn't rest there. Questioning of Mr Drury (who, as it happens, is a former president of the Treasury Board) revealed that the budget of the branch could not be identified separately in the estimates, but instead was spread about in such a way as to make it impossible for MPs to find out how much had been spent

More about spying and the NRC

from David Spurgeon, Ottawa

on this sophisticated form of spying (the branch monitors electronics data signals and analyses them).

Mr Drury defended the procedure by saying it was necessary in order to make it difficult for Canada's enemies to learn about the operation. "The counter-intelligence movement . . .", he said, "must, in order to be effective, itself be under cover . . . To take a popular analogy, this is a bit like engaging in a poker game in which one man discloses his hand by putting it on the table and the other keeps his hidden and then proceeds to bet."

Walter Baker, Progressive Conservative MP, Ottawa, was disturbed because, as he said: "Members are to find out about security organisations by accident" (the matter came up in a CBC television programme last year) "and then be denied any opportunity to

ask questions about the scope and purpose of such activities or the amount of money to be spent on them."

It would be comforting to accept assurances that everything being done in the name of security is fit and proper, but recent history in other countries suggested otherwise, he said.

Mr Drury admitted that the need for secrecy presents democracies with a problem, but the problem was not peculiar to Canada. "The problem, from the administrative view, is how to seek [this] parliamentary approval [for security expenditures] without disclosing in a comprehensible and clear way what is being done, and how."

In the past, said Mr Drury, when members of the House felt they must know what was being done, they had been informed *in camera*.

To which Stanley Knowles, New Democratic Party MP from Winnipeg, replied: "The last *in camera* session of this House of Commons that I can remember took place in 1944." Mr Knowles said it is open to the government to keep things secret, but quite a different matter to distribute items through a number of estimates in order to distort the picture given to Parliament of how money is being spent.

The Speaker concluded the session by saying that the "question is extremely important and complex, and I would like to look at it carefully." In view of the fact that Mr Drury had also offered to tell the ministers what was involved *in camera*, "it would perhaps be best to ruminate over the Easter break and see what might develop." □

Letter from Japan

from Yoshinobu Kakiuchi, Tokyo

Two new inter-university research institutes, the High Energy Physics Institute and the Institute for Molecular Science, have recently been established in Japan. The first is located at Tsukuba some 35 miles from Tokyo. The place is known as an academic town, the intention being that it should centre on universities and various research institutes. The High Energy Physics Institute was the first institution to be brought into the area, followed by the Pollution Research Institute and the Institute for Inorganic Materials, both of which belong to the

An interesting footnote was to be found in a series of recently published letters from Dr C. J. Mackenzie, acting president of the NRC from 1939-1943, to General A. G. L. McNaughton, the president, who was on leave of absence as commander of Canada's field forces. (*The Mackenzie-McNaughton Wartime Letters*, edit. by Thistle, M., with introduction and epilogue by Mackenzie, C. J., University of Toronto Press, 1975).

In one of these letters, dated December 6, 1941, Dr Mackenzie said: "There is one very interesting but very secret development which only one or two of us know about. We have organised under the research council a section on cryptography which has succeeded in breaking down codes and cyphers and doing a really good job. It seems an unusual activity for the research council but the intelligence officers of the three services, the Mounted Police and the officers of External Affairs asked me if we could organise such a unit, as they thought we could probably do it easier and keep

it under cover better than in any other way . . . We have an associate committee consisting of the intelligence officers of the three services, the RCMP, a member from External Affairs and myself. We have an expert cryptographer from England and I look after the business arrangements, finances, etc . . . We got into it in the first instance because our facilities were such that we could start a unit in a modest way to see whether or not it was a practicable thing to do.

"My feeling was that after we had done the organising and got the staff trained, an official unit could be set up by the government to operate under one of the services or External Affairs. After six months of trial, which a decision had to be made as to how it should continue, all of the members were most insistent that they would prefer it to be carried on under our auspices and that will be done, for a while at least. It is pretty far from research but it is a most important and useful service."

Science and Technology Agency. And a new national university (Tsukuba University), based on various new attempts to break with university tradition, has just started activities there.

The Institute for Molecular Science is to be located at Okazaki 190 miles to the west of Tokyo, where it is also planned to build two other new institutes devoted to the life sciences.

The idea of the inter-university institute has been nurtured in Japan for some time. Soon after the Second World War, Japanese scientists began to think of establishing forums where they could exchange their ideas and cooperate in research of common interest. Nuclear scientists, for example, wanted to build an accelerator for nuclear research, but the cost seemed too large to be borne by a single university at that time. Therefore, the facility was supposed to be shared by researchers all over Japan. Traditionally, however, the national universities receive money from the government individually, which made it rather difficult to set up facilities to be shared on an entirely equal basis by several of them. The compromise was that institutes should be affiliated to a particular university, which is made responsible for its operation; but the facilities are available to the research scientists outside that university. At present there are more than a dozen of these so-called "institutes for joint use".

The establishment of these institutes raises a series of interesting issues, and naturally there are some difficulties. Universities in the main remain rather closed in structure, and the institutes for joint use are no exception. They usually open their doors only to scientists closely related to the research they cover, and this often causes friction. Of course, it is not impossible to promote inter-institutional joint projects, as is exemplified by the joint programme set up by the Institute for Nuclear Study and the Institute for Solid State Physics, both of which are affiliated to the University of Tokyo. There had been a good deal of spectroscopic work with electron synchrotron radiation in the past, but the spectroscopists involved wanted to build a storage ring to provide increased energy and radiation intensity. The ring has been successfully tested under the supervision of Professor Taizo Sasaki, of the University of Tokyo.

To escape more completely from the university strait-jacket, the idea of inter-university research institutes on a national basis, completely detached from the existing universities but still keeping a university's essential characteristics, were developed.

The Institute for High Energy Physics, for example, was established in 1971, at the recommendation of the



THE picture of young anacondas is taken from the Annual Report of the Zoological Society of London for 1974. In its annual report the Zoo records a rise of expenditure of 16% over last year, mainly on salaries and animal feeding stuffs. Unfortunately this coincided in

1974 with a fall in the number of visitors to both London and Whipsnade. But the numbers picked up again, apparently, after the arrival of the two giant pandas from Peking as a gift from the Chinese government; they arrived last September in a blaze of publicity.

Science Council of Japan, the advisory body to the Prime Minister's Office on scientific affairs. The recommendation was originally made as early as 1962, on the basis of extensive studies of a possible proton synchrotron. The project was investigated in detail and was finally authorised by the Ministry of Education. This synchrotron produces energetic protons in two steps—the booster ring accelerates them to 500 MeV and the main ring (108 m in diameter) brings them up to 8–12 GeV. The five-year plan presented to start with is to be modified to a seven-year plan, with an increase in the total budget from about £10 million to about £15 million.

According to Professor Shigeki Suwa, the director of the institute, there has been plenty of cooperation between scientists and engineers in the design and the construction of the accelerator. He also says that the institute is keen to open the way for technicians to achieve better positions in the institute through promotion. A storage ring of the colliding beam type, equipped with

super-conducting electromagnets, is still on the drawing board, but is being designed to produce a 70-GeV proton beam. Preliminary testing of the super-conducting wires is progressing satisfactorily.

The building of The Institute for Molecular Science has just been started, and Professor Hiroo Inokuchi, of the University of Tokyo, is now responsible for supervising the preparation of its activities. The setting up of this institute was also recommended by the Science Council of Japan (in 1965) and it is scheduled to cover five major fields of research, ranging from molecular theory to cooperative molecular phenomena.

This institute will be administered along similar lines to the Institute for High Energy Physics, and a development worth mentioning is the establishment of a "technical development section", responsible for the design and development of new research instruments. The original five-year plan for the institute is covered by a total budget of some £6 million. □



Sergei Kovalev: arrested in December

Moscow scientists under pressure

from Vera Rich, London

THE arrest and harassment of members of the Moscow group of Amnesty International highlights yet again both the involvement of a number of leading Soviet scientists with problems of human rights and the extreme dis-favour with which these activities are viewed by the authorities.

Physicist Andrei Tverdokhlebov, the secretary of the group, was arrested in Moscow on April 18, on his way to work. The same day, the Ukrainian science-fiction writer Mykola Rudenko, a postal "associate member" of the group, was arrested in Kiev.

Another prominent member of the group, Dr Sergei Kovalev was arrested last December, as part of a KGB operation against the *samizdat* journal "The Chronicle of the Lithuanian Catholic Church". Dr Kovalev has since been charged with "anti-Soviet agitation and propaganda".

Other members of the Amnesty group, including the Chairman, cyberneticist Valentin Turchin, and Vladimir Albrekht, a mathematician, have had their apartments searched, and documents, including Amnesty material, confiscated.

The Moscow group of Amnesty International was recognised by the International Executive Committee of Amnesty last September. Following the usual procedure, the Secretariat assigned the new group three case-sheets, referring to prisoners of conscience in Sri Lanka, Yugoslavia and Spain respectively. The group as such, had no connection with the internal affairs of the Soviet Union, in accordance with the policy of Amnesty International which does not allow any of its groups to concern itself with prisoners in its own country.

Although certain of the members of

the group, have, as private individuals, also been active as regards the defence of human rights within the Soviet Union (Tverdokhlebov and Turchin have, on occasion, been co-signatories to the "open letters" for Dr Andrei Sakharov), it seems clear that it is not only their actions as private individuals, but also the existence of the Amnesty group which has earned official dis-favour. The latest reports from Kiev indicate that although Rudenko has been released, his continued freedom is conditional; he has had to sign a promise not to leave Kiev, has been warned that he is awaiting further investigation and trial, and that his Amnesty activities "compound his crime" against the Soviet Union.

A statement from Academician Andrei Sakharov, addressed to the General Secretary of Amnesty International and to the "international public", described the arrests and harassments as "an affront to international public opinion and an attack against legality and democratic and humane principles to which this organisation and its members in the USSR invariably adhere."

Soviet space achievements celebrated

In spite of the recent setbacks to the manned Soyuz programme, Soviet Cosmonautics Day, April 12, was celebrated with the usual progress report on Soviet achievements. Although, on the practical side, the festival was marked only by the launch of that most routine of Soviet spacecraft, a Kosmos (number 726), this deficiency was more than offset by the official and quasi-official speeches.

At a celebration meeting held in the Central Theatre of the Soviet Army in Moscow, Academician Boris M. Petrov spoke of the year's work in tones of unqualified achievement. On such an auspicious day, nothing was said of the problems with the Soyuz-Salyut projects. The stress was entirely on the potentialities of the Salyut programme "for long term experiments in space with human participation".

Special emphasis, too, was placed on the expansion of Soviet participation in international space projects. "Successful cooperation" is at present going forward between the Soviet Union and the USA, France and India, and this co-operation on "major projects" will be extended during 1975 to include Sweden. The Interkosmos (joint COMECON) programme also progresses steadily, the latest satellite in the series being Interkosmos 13.

Within the purely Soviet framework, more than 50 Orbita communications satellites have now been launched, and a new one is planned to bring television to the workers on the Baikal-Amur Railway (one of the present major Soviet prestige engineering projects).

Although the official speeches at such a celebration must, of necessity, be laudatory, Academician Petrov's plaudits are, in fact, substantiated by a year of solid routine achievement. Although the Soyuz setbacks have captured the headlines, valuable preparatory work has gone forward on the Soyuz-Apollo plans (the major difficulty still, apparently, being one of linguistics). The Franco-Soviet "Araks" experiment, operating from magnetically antipodal bases in the Arctic and the Kerguelen Islands, is proceeding steadily with its programme of investigations of the terrestrial magnetic field. Although unfavourable meteorological conditions in the Antarctic have made it impossible, to date, to carry out the main part of the experiment—the injection of electrons into the magnetosphere to create an artificial aurora—some valuable, if routine, observations of the natural aurora have been carried out. Interkosmos 13, with its payload of Czech and Soviet instruments, was likewise devoted especially to magnetospheric and polar ionospheric research, a programme which is also being supported by a team on the drifting ice-flow polar station North Pole 22. A few days after the celebrations, on April 19, the first Indian satellite (named Ariabata after a fifth-century mathematician) was successfully placed in orbit from a Soviet carrier, achieving, *inter alia*, the distinction of being the heaviest first satellite of any country (360 kg).

Cosmonautics Day is, however, not only the time for an annual stock-taking of Soviet space achievements; it is also an occasion for press speculation and "informed" interviews on future plans.

Much of this material is necessary speculative and non-committal. "To prove, for example that there is not and was not life on Mars would be no less important than to find life there", is a typical comment of this type. Nevertheless, from these "supporting" interviews it would seem that long term planners are at least toying with the possibility of recovering soil specimens from the nearer planets. The combination of a Lunokhod-type vehicle with telecontrolled manipulators and a return craft of the Luna-16 type is considered "very promising" for this purpose. And, with an eye to the more distant future, the Institute of Medico-Biological Problems of the Ministry of Health is already working out ecological recycling and life-support systems for eventual long term space flights. □

DRINKING water throughout the United States is contaminated with trace amounts of organic chemicals, some of which are suspected carcinogens, according to a survey of drinking-water quality in 79 cities. The survey, carried out by the Environmental Protection Agency (EPA), indicates in particular that chloroform is an almost universal contaminant—it was found in samples of drinking water from all 79 cities, in amounts ranging from 'barely detectable' to 311 parts per billion.

Moreover, a more intensive survey of drinking water in five cities—Miami, Seattle, Ottumwa, Philadelphia and Cincinnati—has turned up evidence of a wide range of chemicals in the water supply. Thirty-five different organic chemicals have been detected in Miami's drinking water, for example, and thirty-six have been found in Philadelphia's.

Just how great a hazard to health those contaminants may be is a matter of some debate, but Russell Train, the Administrator of the EPA, said last week that "even at these low levels, the chemicals are a matter of some concern". The National Academy of Sciences is now evaluating the health hazards, and a special advisory committee has been established by the EPA to help determine national standards for drinking-water supplies.

The EPA survey looked specifically for six different chemicals in drinking water — chloroform, bromodichloromethane, dibromochloromethane, 1,2-dichloroethane, bromoform and carbon tetrachloride. Chloroform was the most ubiquitous, but bromodichloromethane and dibromochloromethane were also present in almost all the samples tested.

Officials of the EPA have expressed surprise at the widespread occurrence of the contaminants, and Train summed up the findings thus: "Our basic conclusion ... is that the problem of organic chemicals in public water supply systems exists throughout the country."

The survey does not identify the sources of the contaminants but there is strong evidence that the chlorination process, which is the most widely used purification method for drinking water, may itself be responsible. A survey of raw, untreated water showed no trace of the six chemicals in 30 of the cities and extremely low concentrations in the other 49. But at least one of the chemicals was present in every sample of treated water that was tested.

The EPA's findings are likely to rekindle the smouldering debate about whether or not there is a safe level of exposure to carcinogens below which no health hazards are encountered. It should be noted, however, that the

food and drug laws in the United States are based on the assumption that there is no such no-effect level, since they specify that no chemical which raises cancer in test animals can be added to foodstuffs. The extension of that concept to drinking water would, however, prove difficult, for Train noted last week that although several promising processes for water treatment are being developed, "we simply do not have a single proven method for dealing with all aspects of the organics problem".

The immediate effect of the EPA's

Washington seen

by Colin Norman



findings is likely to be a boom in the market for bottled water, but tests by both the EPA and the Food and Drug Administration have shown in the past that many varieties of bottled water are no 'purer' than tap water.

● The job market for scientists and engineers who have PhD degrees is likely to deteriorate during the next 10 years, according to a survey soon to be released by the National Science Foundation (NSF). According to projections of the likely output from graduate departments of universities in the United States, there will be between 375,000 and 400,000 scientists and engineers with PhDs by 1985, but only about 295,000 are likely to be employed in jobs related to science and engineering.

Those predictions "indicate a trend toward increasing imbalances between supply and utilisation ... of science and engineering doctorates, possibly in some outright unemployment", the report states. But it adds that although the magnitude of the unemployment is difficult to project, it "is expected to be relatively small since individuals with doctorate education are likely to find some sort of employment—possibly in non-science and engineering activities or in underutilisation of their training".

The NSF's projections indicate that the widest divergence between supply and demand for PhDs is likely to be found in the social sciences, whereas the life sciences are likely to be almost in balance.

A prime reason for the deteriorating

job market is the fact that there is expected to be a decrease in enrolment in universities and four-year colleges, which are traditionally the biggest employers of science PhDs. Consequently, the projections indicate that a smaller proportion of PhDs will be engaged in academic research and development in 1985, and that as many as 20% will not be involved with science and engineering at all.

In fact, the projections suggest that almost half of all new job openings for PhD scientists and engineers in the next decade will be in activities unrelated to research and development. The report suggests that such a shift "has major educational implications for institutions as well as for students".

● President Ford has at last signed an executive order to set the seal on the ratification by the United States of the Geneva Protocol, which outlaws the first use in war of chemical weapons. The executive order, which establishes a "national policy" under which the United States has renounced the first use of herbicides and riot control agents in war, had been bottled up for almost three months in the Justice Department, and formal ratification of the protocol by the United States was being held up until Ford signed it. The order, in short, allows the Administration to hang on to its belief that herbicides and riot control agents are not strictly covered by the protocol, but the United States has renounced their first use anyway.

● The Energy Research and Development Administration (ERDA) has backed off from a plan to store radioactive waste material from nuclear power plants in temporary above-ground storage facilities, and has decided that the whole matter requires more study. The move, announced last week by ERDA Administrator Dr Robert Seamans, follows widespread criticism of the plan by a number of environmental organisations. Although the temporary storage scheme has not been dropped entirely, ERDA may well move straight to a permanent disposal operation, consisting of burying the wastes deep in a salt mine. A promising site is under study in New Mexico, but it will take many years to carry out tests there. The original idea was to store the waste material in temporary facilities until a permanent disposal site had been found and tested, and ERDA had asked Congress for \$5 million to start constructing the above-ground facilities next year. Seamans said, in a letter to the Joint Committee on Atomic Energy, that no decision would be made until 1976 about how the waste disposal operation should proceed.

correspondence

Soviet Jews

SIR,—The wave of repressions against Jews seeking permission to emigrate to Israel has recently increased considerably in the USSR.

We scientists, who are being forcibly kept in the USSR against our wills, wish to express our opinion about the present deteriorating situation. We realise that the Soviet authorities now respond to the sympathy and public opinion of the West, and especially of the USA, in terms of what they can gain politically and economically. The Soviet government has found that the West reacts actively to the voices of the persecuted who are deprived of elementary human rights; they therefore try to deprive these people of their voice and to turn them into obedient instruments of Soviet policy.

So the main attack is directed against people whose protests are considered by the authorities to be the most undesirable. Thus Nashpits and Tsitlenok are now charged with participating in a 12-second-long demonstration of protest against the refusal of the Soviet government to issue visas to them. As recently as three years ago such action would have, at the worst, led to 15 days detention for "disturbance of public order".

The other participants in this demonstration were warned that if they set a foot wrong again their fate will be the same as that of Nashpits and Tsitlenok (now sentenced to five years' exile).

The magazine *Jews in the USSR*, created by Professor Voronel and dedicated to the scientific analysis of different aspects of the cultural life of Jews, was claimed at the trial of the writer Vladimir Maramzin to be against the law and anti-Soviet.

Nowadays, also, participants in the scientific seminar founded by Professor Voronel three years ago are persecuted severely.

● The present leader of the seminar, Mark Azbel, was recently told by the KGB that the important thing is not that he is a professor of physics, but that he is a lieutenant in the military reserves and subject to call-up for military service. It is ironic that a man aged 43, who has never been called up, should now, after 2½ years of waiting for a visa and 3 months in hospital, be needed in the military camps.

● Recently Dr Viktor Brailovskii was called to the KGB where he was threatened with the charge of "insulting a

policeman" during a demonstration on February 24. But he was not involved in the demonstration.

● Dr Aleksandr Lunts was warned by the KGB that he can be charged as a traitor to the Motherland.

● In Kharkov unknown persons tried to break into the flat of Professor Leonid Gerber.

● Dr Mikhail Shepelev received seven summonses to military offices and a summons to appear before the police for so-called "parasitism" (being unemployed).

● Eltan Finkelshtein has been called to the military offices again and again.

● After being fired from his job, Dr Vainer was ordered to begin work in 15 days. But as it is impossible for a Jewish *refusnik* to obtain work in his profession, he will have to take a menial job.

● Dr Mikhail Mikiyinskii and Dr Eugenir Yakir are charged with parasitism as well, in spite of the fact that they were dismissed from their previous jobs.

So, although nobody has been directly charged for participating in the seminar, it now seems too dangerous to be involved in it. And it is the only means of scientific contact for 8 professors, 24 doctors and 18 bachelors of science from eight cities.

The scientists are persecuted for scientific activities as they were in the Middle Ages. This is the true picture of KGB activities to stop the voices of Aliya (the movement for the repatriation of Jews to Israel).

It is hard to predict what will happen if the authorities achieve their aim. If protests cease, the government will be able to say that people no longer want to emigrate.

Whether Russian Jews will play a further part in changing the relationship between the USSR and the USA, and whether they will continue to be repressed is an open question; in any case, if their voices are silenced, the Soviet government would be quite free in its suppression of emigration.

We have too few opportunities for preventing such a sequence of events. We do not know how to convince the Soviet authorities that the humane attitude towards emigration is the only possible way in our time.

It is now abundantly clear that the mass attack against Soviet scientists who participate in Professor Mark Azbel's seminar has been instigated by the KGB in preparation for the 250th

anniversary of the Academy of Sciences of the USSR. (The anniversary session was unexpectedly postponed last year because of elections to the Supreme Soviet.)

We are so anxious for scientific contact that we will be happy to speak at and to listen to as many lectures as possible. But such things are impossible, according to KGB logic. So the spirit of the participants in the seminar must be broken before the Anniversary.

We ask all people who are interested in helping the persecuted to help us—to raise their voices in defence of emigration, in defence of our natural human rights.

MARK AZBEL

VENYAMIN FAIN

ILYA PYATETSKII-SHAPIO

VIKTOR BRAILOVSKY



... it is interesting to note that reports from Sweden and Norway state that during the night of March 29–30 last, a heavy rain of ashes or sand took place from the west coast of Norway to the Swedish frontier; the whole of the country was covered with grey dust to such an extent that from a pint of snow more than a tablespoonful of residue was left after the snow had melted. Some chemists of Christiania have examined the ashes, and one of them, Prof. Waage, states that the dust consists of little, irregular, but sharp-edged grains, almost all colourless—some few are of brown colour—and they consist principally of silicates. Acids extract some lime, iron, and alumina from their powder. The professor thinks it likely that the dust originates from an eruption in Iceland. This view is confirmed by a mineralogical investigation made on another sample of the dust at the Christiania University, by Profs. Kjerulf and Fearnley; they recognised the dust to consist of fragments of pumice-stone which is identical with the Hecla pumice-stone. According to Swedish newspapers, some traces of the dust-fall were observed even in the vicinity of Stockholm. Prof. Kjerulf also thinks it highly probable that an eruption took place in Iceland. The distance from the Iceland volcanoes to the Swedish frontier is about the same as that from Mount Etna to the Baltic.

from *Nature*, 11, 515, April 29, 1875.

news and views

The nucleosome: subunit of mammalian chromatin

from Benjamin Lewin

EACH eukaryotic chromosome contains a very long duplex molecule of DNA, five classes of histone protein whose total weight is about equal to that of the DNA, a lesser weight of a somewhat larger number of nonhistone proteins, and a small amount of RNA. Little evidence has been available to show how these components may be organised at the molecular level, although it has for some time been apparent that DNA and protein form a fibre of regular dimensions which is condensed into the very compact structure of the mitotic chromosome and presumably is relatively less tightly folded into interphase chromatin. Two types of chromosome fibre were identified by DuPraw and Bahr¹ in electron microscopy of chromatin prepared by the critical point drying method (drying on a grid from CO₂); the basic thread folded into the human chromosome seemed to be a fibre of 250–300 Å, which in turn was generated by the folding of a fibre of diameter 50–100 Å. Although critical point drying may alter the dimensions of chromatin organisation, this suggested the general concept of a single continuous fibre folded upon itself many times, with more than one level of organisation.

The existence of a regular repeating unit in both interphase chromatin and metaphase chromosomes of Chinese hamster cells was suggested by Pardon *et al.*^{2,3}, whose X-ray diffraction studies suggested the presence of a supercoiled fibre of diameter 100 Å with a pitch of 110–120 Å. By introducing a new method for visualising chromatin structure, Olins and Olins⁴ were able to suggest that the chromosome fibre might have the type of structure typical of beads on a string. Following the procedure first developed by Miller and Beatty⁵, interphase nuclei of rat thymus

or liver or of chicken erythrocytes were diluted into water; the nuclei swell and break open, the contents are fixed in formalin and then centrifuged on to a grid. Spherical particles, called ν bodies, of about 60–80 Å in diameter can then be seen, connected by a thin (15 Å) filament. Individual ν bodies could be visualised in less stretched region of the fibre; a fibre of greater diameter was generated when the ν bodies were more closely packed together.

Histones

Another line of approach to resolving the structure of chromatin has been developed from analysis of histone properties. Histones are separated on acrylamide gels into five distinct classes, which fall into three groups according to the relative contents of the basic amino acids lysine and arginine in which these proteins are rich. Two different nomenclatures have been in use to describe these five classes; since both serve as a reminder of the erratic progress made in separating histones and now seem somewhat less than satisfactory in their relationships, I shall use a third nomenclature which was proposed recently⁶ (see table).

The lysine-rich histone class H1 displays microheterogeneity and consists of several closely related proteins. This class is the most easily removed from chromatin and its removal allows the preparation to be solubilised; several studies of the physical state of chromatin depleted of lysine-rich histones have suggested the tentative conclusion that H1, as the least tightly bound, may be concerned with surface properties, perhaps with holding together a chromosome fibre consisting of the other components⁷.

The two classes of slightly lysine-rich histones, H2A and H2B, show some conservation between species according to the criterion of gel mobility. The two classes of arginine-rich histones, H3 and H4, both show extensive conservation, with virtually identical amino acid sequences between organisms as unrelated as cow and carp (H3) and cow and pea seedling (H4); patterns of modification are similar but differ in detail between species. (The strict con-

servation of sequence makes these the most conserved proteins known.) Such striking suppression of variation suggests that the entire sequence of each of these histones is involved in its function and that this function may be virtually identical in all eukaryotes. A function common to such different species presumably must be structural, so that this implies that there may be common features in the molecular organisation of all eukaryotic chromosomes⁷.

The tendency of histones to aggregate during extraction has been a complicating factor in analysing their function for some time. Kornberg and Thomas⁸ suggested that this aggregation might be due to the denaturing conditions that were employed and therefore extracted histones by a milder method (in 2 M NaCl–50 mM Na acetate). When this histone preparation was fractionated on Sephadex, three groups of proteins could be identified: one peak contained a complex of H4 and H3; another contained a complex of H2A and H2B; H1 did not complex with any other histone. A tetramer containing two molecules of each arginine-rich histone (H3₂–H4₂) was obtained by applying ammonium sulphate precipitation and using a cross-linking agent; little of this tetramer was found when histones were extracted by the more usual acid and solvent procedure. The slightly lysine-rich histones also form an oligomeric complex (H2A–H2B), although its nature could not be so well defined. When the H3₂–H4₂ tetramer was mixed with the H2A–H2B oligomer, no reaction occurred as judged by the use of a cross-linking agent.

When the tetramer and the oligomer were mixed with DNA, however, Kornberg and Thomas found that they formed the same X-ray diffraction pattern as that displayed by chromatin. Based upon these observations, Kornberg⁹ suggested a model for chromatin in which the basic structure is formed by the interaction of DNA with the H3₂–H4₂ tetramer and H2A–H2B oligomer; the lysine-rich histones H1 and the nonhistone proteins do not participate. The equal weights of histone and DNA present in chromatin

Histone nomenclature

	Original schemes		New scheme ⁶
Lysine-rich	f1	Ib	H1
	(f2c)	V	H5
Slightly lysine-rich	f2a2	IIb1	H2A
	f2b	IIb2	H2B
Arginine-rich	f3	III	H3
	f2a1	IV	H4

correspond to about one histone each of the slightly lysine-rich and arginine-rich classes per 100 base pairs, with the lysine-rich H1 being present in only about half the molar amount. Following this equality, Kornberg proposed that the H3₂-H4₂ arginine-rich histone tetramer is associated with two copies each of the slightly lysine-rich histones H2A and H2B and with 200 base pairs of DNA; this forms the basic repeating unit. The lesser amount of H1 would correspond to one protein molecule for each repeating unit. By postulating that the arginine-rich tetramer forms the core of the repeating unit, it is possible to explain why these two histones are the last to be dissociated from DNA by mild extraction procedures and why they are the most conserved; the role of the slightly lysine-rich histones H2A and H2B might be to determine the spacing of the tetramer along the length of the fibre. This would generate a flexible structure, able to fold in the manner that must be necessary for compression within the chromosome.

Nuclease digestion

Support for this model is provided by several experiments which show that nucleases may cleave chromatin at regularly spaced sites, presumably the sites between adjacent repeating units. An early observation was that of Hewish and Burgoyne¹⁰, who found that an endogenous nuclease in rat liver digests the DNA of chromatin when the nuclei are incubated *in vitro* in the presence of calcium and magnesium ions. When the DNA was purified, it formed a series of bands on acrylamide gels, suggesting that sites at regular intervals are available for cleavage. Clark and Felsenfeld¹¹ similarly found that staphylococcal nuclease is able to digest about half the DNA of chromatin to form fragments with an average length of 100–110 base pairs; in a more detailed report, Axel *et al.*¹² digested duck reticulocyte or calf thymus chromatin with this enzyme and found DNA fragments to be produced predominantly in the size classes 45–60 base pairs, 100–130 base pairs, >200 base pairs. Duck reticulocyte chromatin reconstituted *in vitro* from its components gave a somewhat similar result, except that the >200 base pair peak was more pronounced; the source of the DNA proved to be irrelevant, for the duck chromatin proteins were able to confer the same support against degradation upon any DNA.

Particles also have been obtained by digestion of DNA. Sahasrabudhe and Van Holde¹³ found that brief digestion with micrococcal nuclease converted chromatin into very compact particles sedimenting at 12S. Oosterhof, Hozier and Rill¹⁴ more recently have prepared 11S particles by fragmenting calf

thymus chromatin with DNase II; these particles contained DNA of a length of about 400 Å (125 base pairs). Less extended periods of digestion generated DNA in multiples of this length, of 250, 375, or 500 base pairs. Noll¹⁵ found that more than 85% of the DNA of rat liver chromatin can be converted to subunits sedimenting at 11S upon digestion either with micrococcal nuclease or with the endogenous enzyme; milder digestion allows dimers of 16S to be seen. The 11S particle contained DNA of a length of about 205 base pairs; fragments of 405 and 605 base pairs, obtained upon the milder digestion, presumably correspond to dimers and trimers. The buoyant density of the particles of 1.45 g cm⁻³ in CsCl corresponds to a protein:DNA ratio of 0.77; all five histones and some non-histone proteins were present. Some DNA of 170 based pairs in length also was produced, probably as a product of degradation of the 205 base pair fragment, suggesting that shorter pieces of DNA can result from breakage of the basic unit.

In spite of some discrepancies between the size of the particle and the length of DNA contained in it, these experiments all support the general conclusion that there may be a subunit of chromatin which forms a distinct particle and contains a fixed length of DNA.

Electron microscopy

Results supporting the general model for histone-DNA association proposed by Kornberg and confirming that the length of DNA in the repeating unit is about 200 base pairs have been reported by Oudet, Gross-Bellard and Chambon¹⁶. In a direct visualisation of chromatin structure, chromatin of chicken liver was treated with 700 mM NaCl to dissociate the lysine-rich histone class and so give a preparation that is more readily dispersed; centrifugation through a discontinuous glycerol gradient in 600 mM NaCl then gave two fractions of chromatin, one more dense than the other. Both fractions gave the appearance of beads on a string when examined by electron microscopy, with particles of average diameter 128 Å connected by a fibre of 15–25 Å. Oudet *et al.* termed these particles 'nucleosomes', in analogy with the ν bodies; the connecting thread appears to be DNA since it is of an appropriate diameter and disappears after digestion with micrococcal nuclease. The less dense peak contained tightly packed nucleosomes separated by long stretches of free DNA; the denser peak contained only tightly packed nucleosomes. To exclude the possibility that nucleosomes might have been formed by rearrangement of histones in salt solution, selective de-

gradation with trypsin also was used to prepare chromatin depleted of lysine-rich histones; this gave tightly packed nucleosomes of 124 Å connected by a thread of 18–30 Å diameter. To confirm that this structure represents that of chromatin *in vivo*, another technique was used; chicken liver nuclei were lysed directly on to the surface of a water drop on top of a grid. This revealed a very compact structure consisting of tightly packed 130 Å diameter nucleosomes.

By degrading the free DNA of the chromatin preparations that had been depleted of lysine-rich histone, it was possible to obtain strings containing a few nucleosomes, at later times converted to single nucleosomes. The nucleosome seems to sediment at 10.5–12S and represents a spherical particle with an average protein:DNA ratio of 0.97. Gel electrophoresis showed the presence of equal amounts of H2A, H2B, H3 and H4 histones. When the protein of the nucleosomes was digested with proteinase K, the isolated DNA could be visualised by electron microscopy and had an average length of 667 Å (197 base pairs). These results are consistent with the X-ray diffraction studies that suggested a diameter of 100 Å and repeating length of 110–120 Å; differences in hydration may explain the discrepancy in the spacing. The larger diameter of nucleosomes compared with ν bodies probably derives from differences in fixation procedures, for Oudet *et al.* found that 1% formaldehyde reduced the diameter of the particles to 96 Å.

The nucleosomes disappeared, of course, when the four remaining histones were dissociated in 2 M NaCl from chick erythrocyte chromatin that previously had been depleted of lysine-rich histones. When the chromatin was reconstituted by progressive decrease of the salt to 400 mM NaCl, particles of diameter 131 Å formed, connected by a thread of 15–25 Å. The only difference apparent in this preparation compared with those of chromatin was that somewhat greater lengths of free DNA connected the nucleosomes. Adenovirus DNA or phage lambda DNA gave the same structure when reconstituted with the four histones of calf thymus, a strong indication that the sequence of DNA is irrelevant to the formation of this structure. All four histones are essential if nucleosomes are to be reconstituted.

An indication that a regular repeating unit may also be constructed in another way is provided by the recent observation of Lohr and Van Holde¹⁷ that treatment of nuclei of *Saccharomyces cerevisiae* with micrococcal nuclease generates bands of DNA of definite multiple sizes, 135, 275, 400, 520, 650 base pairs. The

behaviour of yeast chromosomes at mitosis differs cytologically from that of chromosomes of higher eukaryotes and their content of histones is smaller: only three histone bands are found on acrylamide gels, H1 seems to be absent and it seems likely that H3 may also be missing. In this case the regularly repeating unit must have components different from those of mammalian chromatin.

The basic unit of mammalian chromatin thus seems to be a complex of diameter about 125 Å; this contains about 200 base pairs of DNA that are compressed about five-fold in length, and possesses two molecules each of histones H2A, H2B, H3 and H4. The DNA presumably is recognised as a polynucleotide duplex chain. The role of each histone within the nucleosome remains to be established; however, it seems likely that application of techniques analogous to those used to investigate the self-assembling ribonucleoprotein structure of the ribosome may prove profitable. That nucleosomes of all higher eukaryotic chromatin may be constructed very similarly is implied by the lack of nucleotide sequence specificity in the DNA component and by Kornberg's proposal that it is the highly conserved arginine-rich histones, H3 and H4, which interact the most intimately with DNA. Presumably the nucleosomes cannot be identical, however, since there may be differences in the slightly lysine-rich histones, H2A and H2B, and it would be interesting to determine the relationship between the mammalian nucleosome and the structure of the repeating unit of yeast, which may lack H3. The relationship between nucleosomes in

mammalian chromatin presumably is established at the molecular level by protein interactions involving the lysine-rich histones and nonhistone proteins; a system in which to investigate the role of lysine-rich histones may be the avian erythrocyte, in which H1 is replaced by a new lysine-rich histone, H5.

One question that is highlighted by these results is the role of modifications (methylation, acetylation, phosphorylation) that histones seem to suffer in the cell, sometimes on a cyclic basis. Modification is species specific; the extent of modification of H3 and H4, for example, varies in those species in which these proteins have been sequenced. There have been speculations that histone modifications might be imposed to allow histones to bind to DNA sequences with sufficient specificity, but the *in vitro* reconstitution and simple repetitive nature of the nucleosome argue against this conclusion. Perhaps modification becomes attractive as an evolutionary mechanism for permitting changes in the histones or parts of histones whose amino acid sequences are strictly conserved; its role remains to be established.

The lack of nucleotide sequence specificity in the architecture of the nucleosome implies that it is with any higher orders of organisation that an explanation must be sought for chromosome ultrastructure. The compression of DNA within the chromosome or interphase chromatin is many times greater, of course, than within the nucleosome subunit. It is therefore important to establish in what manner nucleosomes may be packed together. If lysine-rich (H1) histones are engaged

in some such general role, than it is the nonhistone proteins which must be responsible for variations in ultrastructure, including the differences between euchromatin and heterochromatin that may affect comparatively large chromosome regions, the existence of specific bands in mitotic chromosomes, and the activation of specific genes (presumably transcription could not be accomplished within the nucleosome which would therefore have to be disrupted). Recognition of specific DNA sequences clearly must be accomplished by regulator proteins and the structure of the nucleosome poses an old question in new guise: how are DNA sequences recognised within the nucleoprotein structure?

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What are tumour-specific antigens?

from D. Allen L. Davies

In the spring of 1900 Paul Ehrlich, Director of the Prussian Institute in Frankfurt am Main, addressed the Royal Society with these words: "The idea has already been mooted by v. Dungern, of attacking epithelial new formations, particularly carcinoma, by means of specific 'anti-epithelial sera' ". Incidentally he also mentioned Metchnikoff's hope of attacking the symptoms of old age with anti-phagocyte serum; the latter has not been followed up with any success as far as I know (or was the paper turned down by *Nature*?). The former has been followed up but without the degree of success needed for clinical usefulness. In spite of this there are still generally great hopes for the future success of cancer immunotherapy and for reasons which are easy to grasp.

Today's cancer treatment is almost

wholly by one or more of three approaches: surgery, irradiation and chemotherapy. Though progress and improvements are continually being made in these areas, especially in relation to particular kinds of tumours, nobody believes that in any of them are substantial improvements likely, of such a scale that cancer would no longer constitute a personal threat of disastrous consequences. If tumour-specific antigens are a characteristic feature of all kinds of neoplastic cells, then they provide a marker to serve as the specific target for an immune attack which could be increased, sustained or artificially engendered for therapeutic use. In this case progress of more general applicability can be imagined.

Paul Ehrlich continued thus: "But even if in the immediate future no

great practical success is attained, we must remember that we are only at the beginning of a rational investigation of properties of cells which hitherto have been far too lightly regarded". If over the last 75 years that rational investigation can be said to have been undertaken, what do we have to show for it? We have vast accumulations of data, the conviction that cancer immunotherapy is still a favoured hope for the future, and the impression that the subject is much more complicated than originally envisaged. We could sympathise with Ehrlich if he were now to think that progress has been slow, but some rules have emerged.

There are, perhaps, three taxa: (1) tumour-associated antigens (carcino-embryonic antigen, α -foetoprotein, and so on), (2) those coded in viral genes (which are not necessarily exposed on

the tumour cell surface) and (3) new surface antigens resulting from intervention of abnormal DNA. This last group, but also some of group (2) are attractive targets for immunological attack, but it is not yet decided if all neoplastic cell surfaces have such new antigens. Authors adhering to one school of thought freely refer to 'non-antigenic' tumours; the other school attribute these to instances where the test methods were of inadequate sensitivity. There are certainly stronger and weaker tumour antigens as there are strong and weak histocompatibility (H) antigens. Tumours obey the transplantation rules (except under bizarre experimental conditions) implying that their H antigens are not grossly changed. From the practical point of view the allogeneic situation is most revealing when enhancing alloantiserum blocks the recognition event, abrogating the prompt rejection of a histoincompatible tumour and allowing free growth.

Much early literature bears on H antigen changes on tumour cells (especially in mice) but these studies have not led to any clear guidelines. On page 713 of this issue of *Nature*, Invernizzi and Parmiani compare a non-antigenic spontaneous mouse tumour and an immunogenic carcinogen-induced tumour of the same ($H-2^d$) genotype to show that the latter expresses an H antigen belonging to other ($H-2^b$ and $H-2^k$) mouse strains, and they believe this to be the true nature of the tumour-specific antigen. The suggestion is made that the tumour antigen possibly resulted from "random mutations at the level of histocompatibility genes". It is especially important to know if such a relationship exists between H and tumour antigens because there is something special about H antigens as targets for immune attack. Not only are they of prime importance in allogeneic situations, they are also the principal target for cellular immune attack even between different species. This surprising information is provided by Lindahl and Bach (*Nature*, 254, 607; 1975). The two contributions make good reading for those working on immunotherapeutic approaches, especially as there is a resurgence of interest in tumour-directed antibodies as carriers of drugs (Davies and O'Neill, Eleventh International Cancer Congress, 1974). This is on the basis that the cellular response of the host has failed, that it cannot be replenished from an allogeneic (histoincompatible) source and antibody is not powerful enough, alone, to wreak sufficient damage on the target.

We should beware of generalising to simple rules such as that tumour antigens are derepressed or mutated H anti-

gens. Such a suggestion was made, for example by Martin, Esber, Cotton and Rice (*Br. J. Cancer*, 28, Suppl. 1, 48-61; 1973), but for at least one particular tumour, tumour-specific and H antigens are physically separable molecules (Davies, Baugh, Buckham and Manstone, *Eur. J. Cancer*, 10, 781-786; 1974) and Yeferof and Klein (*Expl Cell. Res.*, 88, 217-244; 1974) show that H antigens and tumour-specific antigens do not co-cap. Aberrant H antigens derived by mutation or derepression should co-cap using suitable H-directed antibody. On the other hand there are cases where, as tumour antigen increases, H antigen decreases (Haywood and McKhann, *J. exp. Med.*, 133, 1171-1187; 1971); and there are other variations on this general theme.

It is a sobering thought that whereas it would be convenient, and even promising from a practical point of view, if H antigens and tumour antigens had the relationships claimed, we do not actually know the natural physiological role of H antigens and their polymorphism. Among about ten possibilities listed at a recent meeting the favoured role was part of a cell surface recognition structure. One might conclude that a career in cancer research is still a safe one, not likely to be interrupted by an actual cure for the disease.

Hedges as relics of ancient woodland

from Peter D. Moore

MANY conservationists, particularly those with a botanical bent, are agreed that hedgerows in Britain act as an important reservoir for wildlife, though some feel that their importance may have been overrated especially as far as birds are concerned (see *Nature*, 249, 514; 1974). Hooper, a leading botanical champion of the hedges, has stated that as many as 50 species of plant could be threatened with local extinction as a consequence of the current agricultural practice of hedgerow removal (in *Flora of a Changing Britain*, edit. by F. Perring, 58; Clarsey, 1970).

Hooper has also pioneered research into the relationship between plant species richness and the age of hedges, finding that the two are generally positively correlated. This fact can, however, be interpreted in two ways; long-established hedges may have had time for invasion by more species, or, alternatively, old hedges may often be relic strips of rich, ancient woodlands. If the former explanation is correct then it would imply that hedges could act as chan-

nels along which plants migrate, and, if this is so, hedges can be regarded as doubly important, since they provide the means by which reinvasion of isolated fragments of woodland can occur.

Pollard (*J. Ecol.*, 61, 343; 1973) coupled a study of hedge floristics in Huntingdonshire and the Soke of Peterborough with an historical analysis of their origins. His results agreed with the findings of Hooper as far as age and richness were concerned, but he also showed that the richest hedges were those which could be traced back through documentary evidence to old woodland edges, often associated with parish boundaries. Some shrubs were entirely confined to woodland relic hedges, such as field maple, *Acer campestre*, dogwood, *Thelycrania sanguinea* and hazel, *Corylus avellana*. This latter finding is rather surprising when one considers the rapidity with which hazel colonised the early post-glacial land surface of north-west Europe (see Deacon, *New Phytol.*, 73, 1055; 1974). The concept of hedgerows as corridors for plant colonisation began to look unlikely.

Helliwell (*Biol. Cons.*, 7, 61; 1975) has dealt this theory a further blow in his work on hedgerows in Shropshire. Association analysis of his site data split first into base-rich and acidic subsets, and subsequently separated off hedges rich in woodland plant species. These are considered to be relic woodland fragments. Of course the use of a classificatory system of analysis renders it inevitable that a strict division of sites should result. But it is surprising that some invasion of modern hedges has not occurred if they do provide migration routes for woodland plants. This would have produced a continuum of richness.

These conclusions in no way deny the value of hedgerows as objects for conservation; it is the emphasis in determining site priority which should be modified in their light. Hedges of considerable antiquity, due to their richness, remain worthy of conservation effort. The case for maintaining recently planted hedges as aids to botanical recolonisation, however, becomes rather tenuous.

The longest-necked lizard?

from Barry Cox

THE Middle Triassic reptile *Tanystropheus* is one of the most peculiar fossils ever found. The adults are up to 6 metres long but with the head and the enormously elongated neck together contributing nearly one half of the



Adult (foreground) and young (top right) *Tanystropheus*, according to Wild. From *Schweiz. paläont. Abh.*, **95**, 1-162; 1974.

length of the animal. Even more surprisingly, there are only 12 neck vertebrae, and most of the elongation is confined to numbers 3-11, some of which are nearly twice the length of the skull.

Tanystropheus has been found in Switzerland, Italy and Germany. A long and fully illustrated account of it, based on 25 more or less complete skeletons, has now been published by Wild (*Schweiz. paläont. Abh.*, **95**, 1-162; 1974). The characters of the skull, and the apparent presence of autotomy joints in the tail, suggest that despite its extreme specialisation, *Tanystropheus* was a very early branch of the lepidosaur (lizard and snake) stock.

Interpreting the anatomy of this reptile in terms of its mode of life obviously presents many intriguing problems. *Tanystropheus* is found in deposits of the Tethys Sea, and a number of adaptations suggest that it was a marine predator. The hind foot is enlarged and fan-shaped; it was probably webbed, and provided the main propulsive force. The forelimbs and neck could have been used to steer the

spindle-shaped body. Wild suggests that the long neck was flexible, like those of plesiosaurs. This seems a reasonable assumption, despite the small number of neck vertebrae, and some of the articulated specimens certainly show a considerable amount of curvature of the neck. The cervical ribs are thin and extremely long, some extending alongside more than four vertebrae, and Wild suggests that they acted as an elastic brace to strengthen the neck. He states that preserved stomach contents show that adult *Tanystropheus* fed on cephalopods and fish, biting and grasping with their simple, conical teeth. Wild points out that the shallow seas around tropical Triassic Europe, with many reefs and lagoons, probably contained a rich fauna.

The young *Tanystropheus* lived a very different life, according to Wild. He states that small specimens of two of the three species are unknown, and that small specimens of the third species (*T. longobardicus*) have never been found in purely marine sediments. Wild points out that specimens of this last species that are less than about two

metres long have three-cusped teeth. Such teeth are found today in some insectivorous lizards and in the marine iguana *Amblyrhynchus*. He then suggests that the young *Tanystropheus* were terrestrial, and that they stretched their long necks upwards to catch insects on the wing! But, though even the smallest specimens have an elongated neck, this would seem to confer little advantage on an insectivore. In any case, the vertebrae of the trunk show no sign of the large neural spines that would be needed for attachment of the large muscles necessary to hoist the heavy neck into the air—and that are, for example, found in sauropod dinosaurs. Perhaps *Amblyrhynchus*, which is a marine herbivore, provides a better clue to the use of the tri-cusped teeth of *Tanystropheus*, whose young may have fed on seaweed in near-shore waters. If so, this peculiar animal, like the crocodiles today, changed its diet during its life, but not its habitat.

Mad hatter's tea party among X-ray sources

from G. T. Bath

THE world of galactic X-ray sources produces a continuing supply of Lewis Carroll characters. Transient, short-lived X-ray sources, which flare up and then disappear (leaving behind not even a grin) are now a well established class. These flaring sources seem to resemble the optical flaring outbursts of novae, but in the X-ray region of the electromagnetic spectrum. The recent discovery of a further flaring source by the Ariel V satellite groups at Mullard Space Science Laboratory and at the University of Birmingham (Ives, Sanford, and Bell Burnell, *Nature*, **254**, 578; 1975; and Eyles, Skinner, Willmore, and Rosenberg, *Nature*, **254**, 577; 1975) has however added a new problem to exercise the theorists' ingenuity.

Until now the periods associated with X-ray sources seemed to be of three types. First, very short-period regular pulses of a few seconds. These are thought to be due to axial rotation of a magnetic neutron star, which is itself a member of a binary system. The radiation in the pulses is then produced by accretion heating of infalling matter at the magnetic poles. The accreted matter is lost by the stellar companion, which is being slowly cannibalised by the gravitational attraction of the binary neutron star component.

This binary structure itself then provides a second period—the period of the binary orbit—which is generally of the order of a few days in the

sources with which we are at present familiar. A third period, which only occurs in the extraordinarily complex system Her X-1, is the 35-day period on which the X-ray radiation in this source seems to be switched on and then switched off, like a regular recurring X-ray flare.

But the new transient source, which was only visible for about ten days, seems according to the Mullard group to have a 6.75-minute period present in the signal. The problem for the theorists will now be to fit such a period into a binary, mass transfer model. If it is the rotation period of the accreting star it would be extremely, and indeed uniquely, long. If it is the binary orbital period then it would be extremely, and excitingly, short—demanding a very small size for the companion as the source of accreted material. But perhaps, like the 35 day on-off period of Her X-1, it will require a completely different and

individual explanation. If other X-ray sources can be taken as an example it will not be long before a plethora of theories are produced trying to account for this totally unexpected new observation by the Mullard group.

Two recently published optical observations of the X-ray source Cen X-3 by Petro and by Osmer, Hiltner and Whelan (*Astrophys. J.*, **195**, 705 and 709; 1975) add a note of caution and sobriety to the world of X-ray sources. The first of these is a series of photoelectric observations of the variations in brightness of the Cen X-3 binary system about its orbit. The second is a study of the spectral type of the optical, mass-losing star. Both conclude that those values for the masses of X-ray binary stars which in the past have been determined on the basis of fitting the spectral type (that is, the appearance of the star's spectrum) and the luminosity (the star's intrinsic

brightness) to theoretical stellar models can be seriously in error. This is extremely important in the present search for black holes in X-ray sources. For it has been claimed that the masses of the compact X-ray sources in several systems must be greater than the theoretical maximum possible mass for a neutron star. But these two papers demonstrate conclusively that one must be extremely careful in determining the orbital parameters of such X-ray binary systems. Simple-minded arguments based on our general understanding of single-star structure may be seriously in error when applied to the pathological X-ray binaries.

Nonetheless, this work on the Cen X-3 system does not dispose of the problem presented by the system Cyg X-1, in which the X-ray source is almost certainly more massive than the critical neutron star mass. Like the grin of the Cheshire cat this conclusion does not seem to want to fade away. The evidence for the existence of a black hole in the Cyg X-1 system is still, as yet, neither substantial enough to confound the sceptics, nor weak enough for them to dismiss it. Demonstration using the techniques of these two papers that Cyg X-1 was not a unique system would have helped in resolving this critically important issue. Now we must wait either for the sceptics themselves to fade away, or for further dramatic news from present, and future, X-ray satellites.

More from Ariel V

by John Gribbin

FURTHER early results from Ariel V were reported at a press conference on April 17, organised by the Science Research Council to coincide with the publication of the papers describing the source dubbed 'Cen Xmas' (*Nature*, **254**, 577 and 578; 1975). The UK satellite has in fact already produced two important results apart from the Cen Xmas source. The first is a general comparison with the Uhuru surveys: so far, with 30% of the first Ariel V survey completed at a sensitivity 3 to 5 times better than the Uhuru 1971-2 surveys, it seems that 16 of the Uhuru sources have disappeared in the past few years. Nineteen new sources have already been found, one (the brightest for a time) being a transient with a lifetime of only a few weeks. This evidence for a changing X-ray sky highlights the need for Ariel V (and its successors) to continue sky survey programmes.

The second major news item came from Peter Willmore (now at University of Birmingham) who announced that an investigation of the galactic centre region showed a bright X-ray source in February, and that this source had definitely not been detectable as recently as November 1974. This might not seem too remarkable in view of the frequency with which other sources seem to be popping in and out of view, but of course the galactic centre is a particularly interesting region where events that we can only observe through a dust veil,

darkly, seem to be happening at many wavelengths.

The most up to the minute news came from Ken Pounds of the University of Leicester, reporting a second Leicester experiment which "within the past few days" has "shown positive evidence for spectral lines near 6.6 keV and 2.1 keV" in the supernova remnants Cas A and Tycho's Nova. Claims in a release distributed at the conference that "if confirmed, these will be the first positively identified X-ray lines from a cosmic source" may seem a little exaggerated to those who remember the days when Sco X-1 was the only known non-solar cosmic X-ray source; it depends, perhaps, on the definition of "positively identified", but there has long been quite convincing evidence that iron lines have been detected on occasion in the flux from Sco X-1.

But that does not detract from the importance of these observations, which suggest an abundance of heavy elements in the remnants several times greater than the cosmic norm, nicely in agreement with the generally accepted view that all elements except hydrogen and helium are synthesised in stars and distributed through the galaxies by nova and supernova events.

Where next for Ariel V? Peter Sanford (Mullard Space Science Laboratory) says that the satellite should have a useful life of a couple of years, so the story is far from over.

Meteors by television

from David W. Hughes

NEW techniques are always welcome and meteor scientists have acquired one: television. The time-honoured method of observing meteors—standing outside on a clear moonless night and gazing intently at the sky—still provides many useful results. About ten meteors per hour can be seen; the eye can detect meteors down to about the fifth magnitude (produced by particles with masses in excess of 0.1 g) in the direct line of vision. The field of view is a cone with an apex angle of around 120° but sensitivity to the meteor is strongly dependent on its position in the field of view: the possibility of a meteor far from the direct line of vision being seen decreases drastically with decreasing magnitude.

This field of view restriction can be alleviated by using a camera such as the Baker Super-Schmidt meteor camera. This has a low *f* number (0.8), a circular field of view 55° in diameter, uses high speed film and can photograph

down to fourth magnitude meteors. If used in conjunction with rotating shutters the meteor velocity can be calculated. Persistent glowing trains left by bright meteors have been studied using an objective shutter and taking successive exposures at two-second intervals, slewing the camera by 0.3° after each exposure to separate the images.

What advantages does television have over these older techniques? First, it is considerably more sensitive; Clifton reported in the *Journal of Geophysical Research* (78, 6511; 1973) that magnitude nine meteors can be detected using his SEC (secondary electron conduction) vidicon system coupled to a single stage image intensifier. Up to 180 meteors per hour were seen in a field of view 13° by 16° . An image orthicon system which could detect stars of the same limiting magnitude (11th) only recorded half this rate because it was less able to detect fast moving objects.

The second advantage is the high degree of time resolution. The television cameras effectively take a photograph of the meteor every $1/25$ th of a second. The video recording thus contains a nearly complete record of the train's movement, its growth and subsequent decay and the light intensity as a function of distance along the train. A comparison of the $1/25$ th-second time interval with the two-second interval of the slewed Super-Schmidt underlines the importance of the new technique. If two spaced cameras can be used a light intensity against height profile can easily be obtained for all meteors detected by both. A large number of these profiles, for meteors of differing velocities and from different meteor showers, should help sort out meteor ablation, fragmentation and structure problems.

More results of television observations have been reported by Hawkes and Jones (*Mon. Not. R. Astr. Soc.*, 170, 363; 1975). They compared the inferred mass distribution and incident flux of the observed meteors with values obtained previously using radar techniques. Radar can detect trains produced by meteoroids of similar mass (that is $>10^{-4}$ g) but measures the electrons produced by meteoroid ablation and not the de-exciting atoms and molecules. Radar suffers from an awkward selection bias inasmuch as the initial radius of the train and its speedy radial diffusion can lead to destructive interference in the reflected beam. As both initial radius and diffusion are height dependent the meteors which ablate high up could go undetected. There is also the underdense-overdense radio reflection transition around third magnitude. Here the equations governing reflected power change. Changes in flux and mass distribution index around

third magnitude could be caused by transition problems and not be inherent in the incident flux. Fortunately the television system does not suffer from any of these drawbacks. Increases in height and range simply decrease the observed light according to the inverse power law. The luminous meteor train being optically thin does not undergo the third magnitude transition, from surface reflection to reflection by all the train electrons, suffered by radar.

Hawkes and Jones conclude that television meteors with magnitude between three and six have a mass distribution index of 2.02, very close to the values obtained by radar in this range. Also the incident flux is, within experimental uncertainty, the same as that observed by radar. It therefore seems that the initial radius bias is of only minor importance in radar back-scatter work, the height of maximum ablation not depending as strongly on mass as previously supposed. The diurnal variation of influx is in qualitative agreement with theory with however a subsidiary maximum around 01 h local time. This supports the original observations of Clifton. Statistical analysis of meteor train lengths indicates that the very faint meteors observed by the television systems (fifth to seventh magnitude) have vertical train lengths of about 6.7 ± 0.7 km, in good agreement with Verniani's work (*J. geophys. Res.*, 78, 8429; 1973) on faint radio meteors—once more indicating that these small meteoroids (mass $\sim 10^{-4}$ g) are fluffy dustballs and not solid and compact pieces of rock.

The television system does have problems. Clifton, assuming all meteors had a spectral class of A0, found that his system detected all meteors brighter than magnitude 6.4, but only 50% of the meteors brighter than 8.1. This problem is also encountered by Hawkes and Jones who find the sensitivity of the television system decreasing as they move out from the centre of the field of view. Even more serious is the velocity dependence of the observations. The fainter the visual meteor the slower it has to go to be detected. The system is therefore biased towards bright fast meteors (the peak train intensity is proportional to the product of the mass and the fourth power of the velocity). But television detection of meteors is here to stay and will be put to many exciting uses. Only about 40 hours of observation are needed to observe enough meteors to sort out the long outstanding problem of the third magnitude transition. And it is possible to observe the whole magnitude range from about 7 up to -1 using television alone, instead of the hotchpotch of underdense and overdense radar, telescopic, photographic and visual techniques used previously.

Status report on the inner planets

by John Gribbin

ON April 11 the Royal Astronomical Society, the Royal Meteorological Society and the Geological Society held a joint meeting for the first time. Though the meeting was entitled 'Mariner 10 Results on Venus and Mercury', it included results on other planets and data obtained from other sources.

DISCUSSING the structure and composition of the clouds of Venus, G. Hunt (Meteorological Office) described the evidence for the presence of sulphuric acid in the cloud tops. In spite of Mariner 10, however, it is still far from clear what lies closer to the surface of the planet; Hunt plumped for a two-layer structure with an upper aerosol layer separated from a deeper cloud layer by a cloud-free region, but confessed that the field is "still wide open" for alternative models.

Some of the greatest interest in the Mariner 10 Venus pictures has centred on the dramatic evidence supporting the view that the Venus atmosphere rotates with a four-day period, producing V-shaped cloud formations. John Hinch (University of Cambridge) was persuaded to bring out of semi-obscurity a two-year-old model of the movement of the Venus atmosphere which, from rather simple assumptions, produces 'predictions' which seem, in the light of Mariner 10, almost too good to be true.

This is basically a 'moving flame' model, in which the heat of the Sun passing over the atmosphere (the moving flame) leads to a thermal tide with resulting circulation. Hinch's lucid discussion was memorable for the smooth way in which some rather unpleasant mathematical modelling was interpreted in physical terms which sounded quite plausible. By assuming a uniform temperature within the atmosphere, large magnitude fluctuations, a thin viscous boundary layer, long wavelength disturbances and a mean field approximation he came out with figures corresponding to a 2 K temperature flux travelling at 7 m s^{-1} leading to a disturbance propagating at 70 m s^{-1} , which corresponds to a five day rotation of the atmosphere (moving, correctly, in the retrograde sense). By comparison with this, the related model of Alan Plumb (Meteorological Office) was intimidatingly mathematical, though his numerical results looked as good as those of Hinch's model.

Jack Meadows (University of Leices-

ter) attempted to explain the evolution of the Venus atmosphere, comparing the process with the evolution of the Earth's atmosphere. The two seem greatly different today, chiefly because of the lack of water on Venus. Meadows pointed out that the difference could be explained in terms of a photo-dissociation process operating strongly on Venus to break water down (through interactions involving carbon dioxide) to oxygen and hydrogen, the latter escaping into space. But he said further that there is no need to invoke this process, and that he would be prepared to accept that Venus has had a predominantly carbon dioxide atmosphere "from the start".

In this model, CO_2 is produced by the effect of heat on carbonates and silicates in the Venus rocks. Several reversible reactions involving these compounds include carbon dioxide as a product, and the amount of CO_2 present would then depend just on the temperature. Once a little atmosphere existed, the greenhouse effect would allow conditions very like those on Venus today to be established, given the boundary condition of the present output of the Sun. Since the Sun is slowly warming up, this suggests an increase in the density of the Venus atmosphere in future. The initial wisp of atmosphere needed to start the process could either come from a burst of volcanism, or slower outgassing.

Venus without its atmosphere must have been much the same as Mercury is today, and J. E. Guest (University College, London) described with the aid of Mariner 10 pictures the surface of Mercury and impact theories of the cratering. Much of this material formed something of a travelogue; for the mixed audience, the most interesting feature of the Mariner 10 pictures is that signs of compressional faulting indicate that the planet has shrunk by one or two kilometres since its formation.

The new studies of the inner planets support Runcorn's attempts to describe Mercury, Venus, Earth, Moon and Mars in one relatively simple model. If Mars and Mercury are both cratered like our Moon, then surely Earth and Venus have been through the same bombardment, providing a clue to events early in the development of the Solar System. If tectonic processes have occurred on Mars (and shrinkage on Mercury) geophysicists may gain a better insight into continental drift on Earth. And if satisfactory models of the Venus and Mars atmospheres are developed, this must help in understanding the workings of our own atmosphere.

As long ago as 1958, the RAS and

RMetSoc held a joint meeting on planetary atmospheres. The way things are going, it certainly will not be a further 17 years before another tripartite meeting about the inner planets is held.

The sea as a chemical system

from Peter S. Liss

The Dahlem Workshop on the Nature of Seawater was held in Berlin on March 10-15, under the chairmanship of Professor E. D. Goldberg (Scripps Institution of Oceanography).

IN comparison with the solutions normally studied by pure chemists, seawater is a very complicated medium. Not only is its ionic strength high ($\sim 0.7 \text{ M}$), but the ions in solution represent virtually all the naturally occurring elements, with concentrations of different elements ranging over at least twelve orders of magnitude. Furthermore, many of the dissolved species are involved in biological processes and reactions at the air-sea and water-sediment interfaces. Pure and marine chemists met at the workshop to discuss techniques and concepts in chemistry which could deepen our understanding of seawater and of chemical reactions occurring in the marine environment.

The ionic strength and composition of seawater are very different from those of the aqueous media normally used for the determination of stability constants. Calculation of the effect of seawater yields only approximate values and for more accurate work, the discussion group which dealt with this topic concluded that it is much better to employ the ionic medium scale, that is, to use seawater (real or artificial) as the reference solution. A self-consistent set of conventions was proposed and its use will allow the development of uniform and thermodynamically rigorous data for seawater equilibria. The new approach has many important implications for marine chemistry, including the redefinition of pH and redox potential, and the use of reference solutions which closely match seawater in ionic composition for pH and ion-selective electrode measurements. It will obviate the need to use single ion activity coefficients and lead to phasing out of the scheme involving 'apparent' stability constants, which has served chemical oceanographers well for many years.

Sample collection is a perennial problem in seawater chemistry and is

especially acute for the sea surface microlayer. Most techniques available at present involve dipping a screen or plate into the water to collect a layer approximately $200 \mu\text{m}$ thick. For the rapid collection of milligram quantities of material, consideration should be given to the technique of foam fractionation in which air or nitrogen is bubbled through the water to create a foam which, because of its large surface-to-volume ratio, will very efficiently scavenge surface-active molecules.

Less than 10% of the dissolved organic matter in seawater has been characterised and an important objective should be the identification of the remainder in representative samples from the deep ocean, above the thermocline and surface microlayer. Techniques are probably available for detailed identification, but these tend to be complex and expensive. Characterisation into broad classes of compounds and major functional groups is simpler and will yield much important information.

Our present knowledge of the particulate fraction in marine waters is rudimentary. For instance, in order to measure size distribution of particles the change in solution conductance due to the presence of the particles is often employed. However, using this method only part of the size spectrum can be examined at any one time and what is really measured is not the particles themselves but a complicated function of the relative conductivities of the particle and the solution. A much more promising technique is to use single-particle scattering counters. These devices, which use laser sources, have already been employed in studies of hydrosols and aerosols.

Though much is known about 'pure' solids (for example the role of electrical double layers, ion exchange reactions, particle-particle interactions), in the marine environment such properties are certainly very substantially altered because the particles have an organic coating. At present very little is known about the properties and composition of such layers, although it should be possible to use techniques such as microelectrophoresis and potentiometric titration to identify some of the major organic functional groups in the coatings, as well as in organic particles more generally.

The goal of the workshop was certainly achieved in a number of areas of seawater chemistry; reported here are only a few of the topics discussed. Where less progress was made it was generally due to the inability of the marine chemists to state the problems in a meaningful way, rather than any reluctance on the part of the chemists to get to grips with the complexity of the sea as a chemical system.

review article

Form and function of cat retinal ganglion cells

W. R. Levick*

Recent explorations of the morphology of retinal neurones, combined with neurophysiological recordings have made it possible to link specific anatomical types with particular physiological classes. At the same time, the relatively complete anatomical mapping of the retina has revealed some bias in the sampling of neurones by electrophysiological techniques.

THE retina has always offered special opportunities to students of the nervous system. It is an extension of the brain conveniently located outside the skull and lining the inside of the back of the eyeball where it is accessible to exploration with a microelectrode under direct visual control. The ganglion cells, which are the retina's output neurones, are spread out as an essentially two-dimensional sheet and constitute the innermost cellular layer of the retina.

The ganglion cell dendritic tree provides the morphologist with a unique opportunity because it is confined to two major dimensions of spread. The pattern of a well stained dendritic tree can thus be directly determined in its entirety when the retina is mounted flat on a slide. The difficulties of assessing three-dimensional structures (thick sections, confusion from overlapping and obliquely running processes) are largely avoided.

The constraints which determine the form of the dendritic tree are not easily comprehended in a representative piece of nervous system but in the retina the sideways spread of the tree strongly conditions how much of the subjacent retinal image is accessible to a particular ganglion cell. There are considerable variations in the spread and mode of branching of different dendritic trees in the inner plexiform layer of the retina and so one is encouraged to expect important relations between structure and function in the spatial domain.

Morphology of retinal ganglion cells

A great deal was known about the appearance of ganglion cells long before their function became accessible to direct investigation. Cajal¹ made superlative analytical use of the Golgi method, which stains only a very small number of the cells actually present, but reveals their form in minute detail (Fig. 1). Cajal was keen to put forward a general morphological plan for all vertebrate retinæ, based on certain characteristic forms of the cells. In the case of the ganglion cells, his classifications were based on: (1) the relative sizes of the cell-bodies (giant; middle-sized; small); (2) the number of layers in which the dendritic tree was arranged (single-layered; bilayered; multilayered; diffuse—that is, no obvious layering); (3) the particular strata of the inner plexiform layer to which the dendritic branches were distributed (first, second, . . . fifth); and (4) the density or sparseness of the dendritic branching pattern. Independent application of these four dimensions could lead to a very large number of morphologically distinct types of ganglion cell. According to Cajal, however, not all of

the possible types were observed in a 'typical' mammalian retina. Taking his descriptions literally, one may count up to 14 classes in the general case. In the cat, there could be still fewer classes because Brown and Major² cast doubt on the generality of Cajal's multistratified inner plexiform layer. They could distinguish only two sublayers.

The interesting recent development in the cat has been the substantial simplification of the morphological classification which has at the same time opened up the possibility of relating the form of ganglion cells to their function as revealed by neurophysiological studies on the responses of single cells. Boycott and Wässle³ achieved their simplified classification by studying the cells in plan view, rather than side on as Cajal had done, and arrived at a qualitative separation into three classes—alpha, beta and gamma cells. The discrimination was based on the recognition of differences in the form of the branching pattern of the dendrites.

Examples of alpha cells are shown in Fig. 2a, and b. They have rather sparsely branched dendrites going relatively straight out radially from a large perikaryon. Figure 2c–e shows beta cells. At equivalent retinal locations they are smaller in all respects than the alpha cells and have more branches per unit area of dendritic field. Most of the gamma cells (Fig. 2f, g) are characterised by small, often oval, cell bodies and a few very thin dendrites branching much less frequently than alpha and beta cells. Gamma cells may be a less homogeneous population than the other two classes. The example of Fig. 2g illustrates a form which may be sufficiently distinctive to constitute a separate morphological type, the delta cells.

Information on the size of axons is important for comparisons with physiological data. Unfortunately, quantitative measurements of diameters are unsatisfactory because of irregular distortions along their length. Qualitatively, Boycott and Wässle³ noted that at equivalent retinal locations the axons of alpha cells were larger than those of beta cells and these in turn were larger than those of gamma cells.

A key result from the analysis of Boycott and Wässle³ was the demonstration of a systematic quantitative gradation in size of alpha cells and beta cells with distance from the centre of the area centralis. The area centralis in the cat is the analogue of the human fovea which corresponds with the centre of gaze. The gradation involved principally the diameter of the field covered by the dendritic tree and to a more limited extent the diameter of the cell body. The recognition that a characteristic morphology may have a systematic quantitative variation with eccentricity has been essential in side-stepping the impasse which would result from rigid adherence to fixed size ranges of cell bodies⁴.

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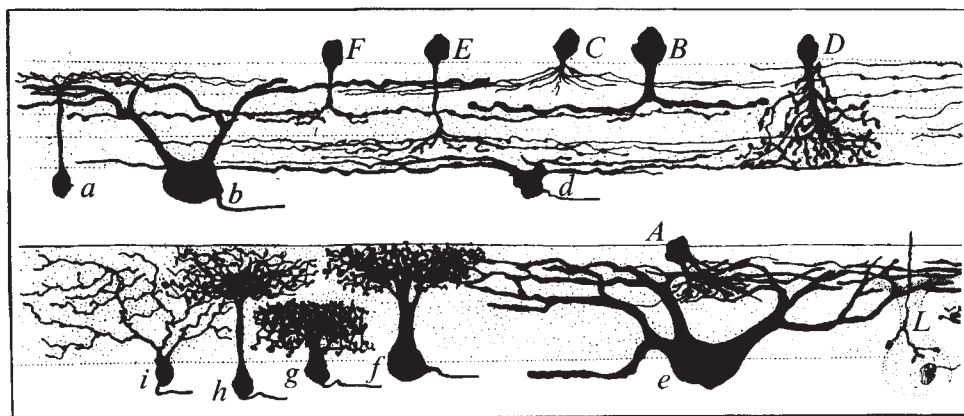


Fig. 1 Ganglion and amacrine cells from the dog stained by the Golgi method as seen in thick sections made perpendicular to the retinal layers. This is a selected part of Plate V in Cajal¹, who did not illustrate his work from the cat, but stated that cat, dog, pig and other mammals were virtually identical. Giant (*b*, *e*), middle-sized (*f*), and small (*a*, *d*, *i*, *h*, *g*) ganglion cells (all the rest are amacrine cells) have their dendritic trees running upwards and spreading out laterally in various fashions in this side-on view. It is interesting to compare *b* and *e* with alpha cells (Fig. 2 *a*, *b*); *f*, *g*, *h* with beta cells (Fig. 2 *c*, *d*, *e*); *a*, *d* with gamma cell (Fig. 2 *f*) and *i* with a delta cell (Fig. 2 *g*), but Cajal unfortunately did not provide scales or relate the cells to eccentricity from the area centralis.

Physiological types of retinal ganglion cells

Cat ganglion cells became accessible to functional testing by the technique introduced by Granit and Svaetichin⁵. The principle was to record the train of impulses which is the output message of a ganglion cell by bringing a delicate, insulated probe very close to the cell body. Rushton⁶ showed neatly that such early recordings represented the activity of individual 'giant' ganglion cells. It was impractical to study the spatial sensitivity of ganglion cells until Talbot and Kuffler⁷ devised a cannulation method for introducing the microelectrode into the intact eyeball, thus preserving the natural imagery. With the application of fine, sharp, electrolyte-filled micropipettes⁸ or tungsten-in-glass electrodes⁹ which penetrate rather than rest on the surface of the retina it has become possible to record from a wide range of ganglion cells.

A key property of ganglion cells is the concentric organisation of their receptive fields. Kuffler^{10,11} showed that an individual ganglion cell was sensitive over a substantial patch of retina (0.8–2 mm), rather as Hartline¹² had found much earlier in the frog. The cat, however, differed from the frog in that the type of responsiveness was not the same throughout the patch. If a ganglion cell responded to a small flashing test spot by a discharge at turn-on for locations near the centre, then the discharge would come at turn-off when the spot was located in an annular surround zone at some distance from the centre. Such a cell would be of the on-centre off-surround type. Off-centre on-surround fields were also found and the two types occurred in about equal numbers.

Classification of cat receptive fields

For many years it was accepted that there were only the two classes of ganglion cell (on-centre off-surround and off-centre on-surround) with a continuum of variation of centre sizes and a preponderance of small-centred cells in the area centralis¹³. Enroth-Cugell and Robson¹⁴, however, showed that the population of concentric receptive fields could be dichotomised according to the nature of the spatial summation of excitation over the receptive field. In one class of cell the processes of spatial summation were linear (X cells) whereas in the other the processes were very nonlinear (Y cells).

Two other dichotomising subdivisions of concentric receptive fields have recently been developed. The sustained-transient⁹ or tonic-phasic¹⁵ dichotomy was based on applying a battery of visual tests, one of which depended on the extent to which the response of a ganglion cell was sustained when the eliciting stimulus was kept steadily on the centre. The brisk-sluggish dichotomy¹⁶ was based on the responsiveness of cells.

Brisk cells, which were encountered about six times as frequently as sluggish, could be induced to generate powerful responses by strong stimulation. The responses of sluggish cells increased far less steeply with stimulus strength and remained relatively feeble regardless of the form or conditions of stimulation.

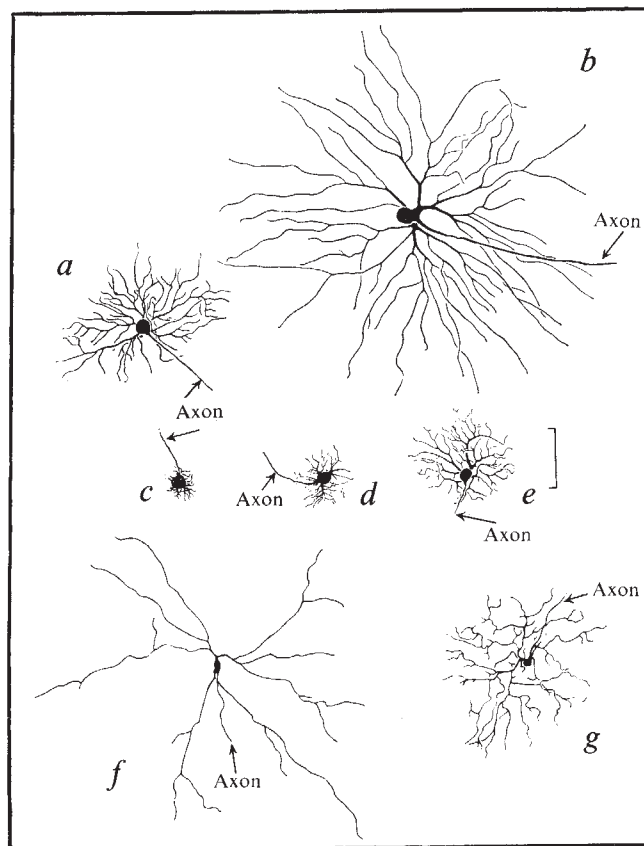


Fig. 2 Golgi-stained ganglion cells from flat-mounts of the cat's retina as seen in plan view (from Boycott and Wässle³, with permission of the authors and the Physiological Society). *a*, *b*: Alpha cells. *c*, *d*, *e*: Beta cells. *f*: Gamma cell. *g*: Delta cell. The cells *a*, *c*, *f* were located at 1.2 mm from the centre of the area centralis, *d*, *g* at 2.9 and 2.8 mm and *b*, *e* at 10.0 mm. All are shown at the same magnification (calibration bar near *e* = 100 μ m).

Table 1 Characteristics of five non-concentric classes

Receptive field types	Morphological identity
1. Concentric centre-surround types (comprising on-centre and off-centre varieties)	
a Brisk	Alpha Beta
(i) Transient	
(ii) Sustained	
b Sluggish	
(i) Transient	Gamma (delta, ...)
(ii) Sustained	
2. Non-concentric	
a Local edge-detector	
b Direction-selective	
c Colour-coded	
d Uniformity-detector	
e Edge-inhibitory off-centre	

Chiefly on the basis of the nature of responses when fine striped patterns moved over the receptive fields, it was argued that brisk-sustained cells were X cells (linear) and brisk-transient cells were Y cells (nonlinear)¹⁶. However, these affinities have not yet been checked with the specific stimuli used by Enroth-Cugell and Robson¹⁴ in the case of sluggish-sustained and sluggish-transient cells. There is a recent tendency to classify cells as X or Y according to the conduction velocity of their axons. This is regrettable because the original principle of classification is interesting in its own right, and we do not know that it coincides with the conduction velocity principle for all types of cell.

Finally, it has gradually emerged¹⁷⁻¹⁹ that there are rarely encountered ganglion cells having receptive fields organised quite differently from the concentric centre-surround type described by Kuffler. The situation has now been studied systematically on a large sample²⁰ and the characteristics of five distinct non-concentric classes described (Table 1). It was suggested^{19,21} that such cells be referred to collectively as 'W cells'. The terminology has, however, become confused since Stone and Fukuda²² now include sluggish concentric centre-surround receptive fields among the W cells, mainly on the ground that they have slowly conducting axons like the non-concentric classes.

Physiology and morphology

The diversity of types of ganglion cells immediately opens the possibility of relating morphological and physiological classes. One approach is to compare the spreads of dendritic trees and the sizes of receptive fields, just as Lettvin *et al.*²³ attempted many years ago in the frog.

The first complication is that the cat receptive fields are composite. Should the comparison be with the total receptive field or only with the central subdivision? Gallego²⁴ pointed out that dendritic fields of giant ganglion cells were much smaller than total receptive fields measured by Kuffler¹¹. Gallego²⁵, Brown and Major² and Dowling and Boycott²⁶ (monkey) argued that the correspondence should be between the dendritic fields and the centre components of receptive fields. It was not until systematic measurements of the extents of receptive field surrounds were made²⁷ that these arguments became more acceptable. It turned out that all but the largest dendritic trees are smaller than the smallest ($\approx 400 \mu\text{m}$ in diameter) total receptive fields. The comparison should therefore focus on dendritic trees and receptive field centres.

The next complication is to decide what is involved in a measurement of centre size since there are systematic discrepancies in the data presented by different investigators^{13-16, 22, 27-29}. Scattered light¹¹ and optical degradation of retinal imagery eliminate the possibility of stimulating individual receptors in isolation in order to determine those which contribute to the centre response. The external test stimulus can be made sharp and tiny but the retinal image cannot. So the experimenter must fall back on stimulus spots of significant size and more or

less indirect methods. Cleland and Levick¹⁶ pointed out that any particular measurement convention implies some assumption about the physiological processes underlying the organisation of receptive fields. There are important qualitative differences in properties between the classes and therefore differences in the nature of the underlying processes. Since these are only partly understood²⁸, one must proceed empirically and not be put off by minor discrepancies. Moreover, one should not necessarily expect to find exact dimensional matching between receptive field centres and dendritic fields. Ganglion cells do not receive input directly from receptors but only through intermediate neurones (bipolar cells, amacrine cells) which themselves have significant lateral spread of their processes.

Two of the physiological classes are compact and dimensionally distinct from each other: the brisk-transient and brisk-sustained classes¹⁶. So are two of the morphological classes: the alpha cells and beta cells³. It is natural to attempt to pair these off. The centre sizes of brisk-transient units are large and match very well the dendritic fields of alpha cells (Fig. 3a), both in the trend with eccentricity as well as in the range of variation at particular eccentricities. The possible identification of these entities is supported by the fact that the axons of brisk-transient units had the shortest antidromic conduction times¹⁶. This implies that they had the largest axons as was observed for alpha cells³.

The identification of brisk-transient units and alpha cells was confirmed more directly by mapping out the centres of

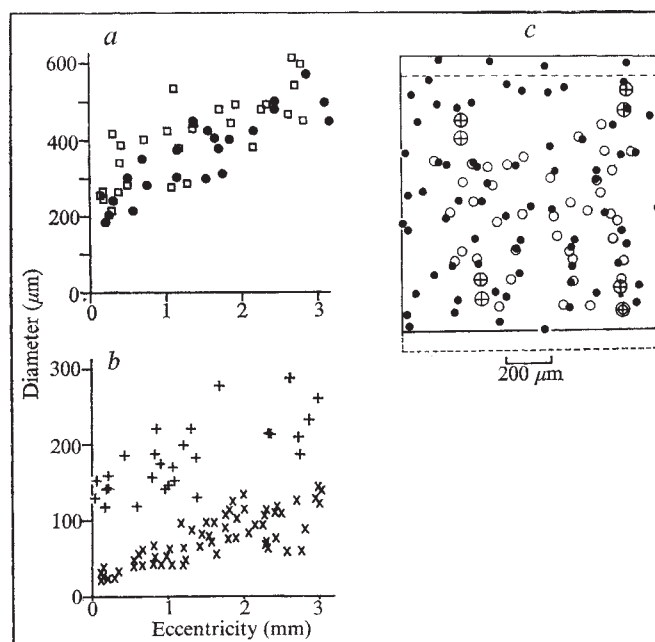


Fig. 3 Comparison of diameters of receptive field centres and dendritic fields of: a, brisk-transient units ($\square$) and α cells ($\bullet$); b, brisk-sustained units ($+$) and β cells ($\times$) (dendritic field data from Boycott and Wässle³, receptive field data from Cleland and Levick¹⁶ with permission of authors and the Physiological Society). The centre sizes were obtained from maps of the receptive fields made with small flashed test spots and show close agreement in a, b. Brisk-sustained centres have the same gradient of size (and similar variation) with eccentricity as beta dendritic fields, but are systematically about $100 \mu\text{m}$ larger at each eccentricity (note the increased vertical scale). c, Comparison of the plotted positions of the geometric centres of the receptive fields of all the brisk-transient units ($\circ$) in a small patch of retina with the position of the cell bodies of all the alpha cells ($\bullet$) in the same piece of retina after mounting and staining with cresyl violet. The four pairs of cross within circle symbols indicate the position of electrolytic lesions made at predetermined sites during recording and later identified histologically. The separation of the members of each pair indicates that a small downward shift of the receptive field map relative to the alpha cell map was required for optimal registration of centres with cells.

the receptive fields of all the brisk-transient cells throughout a small patch of retina. This was identified by lesions and stained with cresyl violet to show the cell bodies of all the ganglion cells present. The distinctive large ones are the alpha cells³⁰ and they agreed in number and position (Fig. 3c) with the brisk-transient receptive fields³¹ to within experimental tolerance. In retrospect, this demonstration supports the identification of dendritic fields with the central component of the receptive fields in the case of the alpha cells.

The comparison¹⁶ of brisk-sustained centres and beta dendritic trees³ (Fig. 3b) is not quite so encouraging. Even though the diameters of both increase gradually with increasing eccentricity, the centres are systematically larger by about 100 μm . The comparable data of Stone and Fukuda²² on centre sizes would provide a better fit, but it should be noted that they used an unusual convention for determining centre size. Support for the identification comes from consideration of the axons: brisk-sustained cells had intermediate antidromic conduction times¹⁶ and beta cells had axons of intermediate size³.

By exclusion the remaining physiological classes (concentric receptive fields of the sluggish kind and the rarely encountered non-concentric receptive fields) may be associated with the morphological gamma (and delta cells.) On both sides there is substantial heterogeneity and little to be gained from dimensional comparisons at the present stage. About the only common feature is the possession of a thin axon and slowly conducted impulses.

Relationship of physiological and histological results

The linking of functional and morphological entities opens up a realm of interrelated data in which the physiological and histological methods play complementary roles. For example, it is comparatively simple to trace the central path of the axon of a ganglion cell physiologically by strategic placement of stimulating electrodes in the brain. Tracing particular axons is a rather difficult feat histologically. On the other hand, it would be an Herculean task to enumerate the brisk-transient cells in the retina by exhaustive physiological exploration, yet it is comparatively simple to count them as alpha cells in a cresyl violet stained flat-mount. There were 6,212 in one whole cat retina³⁰.

Again, microelectrode recordings have long been suspected of giving rather selective access to a heterogeneous neuronal population. For example, brisk-transient receptive fields were encountered on about 10% of recordings in the area centralis and on more than 50% in the periphery¹⁸, yet the histology showed alpha cells to be an approximately constant 3% of the total ganglion cell density throughout the retina³⁰. They are greatly over-represented in physiological recordings. This selectivity, which may be related in some way with the cross sectional area of the cell body, is much harder to assess in the three-dimensional structure of the brain³². The result in the retina is a warning against uncritical acceptance of recording percentages of different classes of neurones as direct indicators of actual numerical preponderance.

As a further example of the interplay of the two approaches, the histology gives us the average density of alpha cells over small regions throughout the retina together with the corresponding dendritic field size. The physiology links the latter to the receptive field centre size which is the significant component for representing the visual field. It is then a simple matter to calculate the extent to which a given point in the visual field is redundantly represented by the set of brisk-transient cells. The answer was a relatively constant, 4.0 to 7.3 in spite of variation of cell density from about 200 mm^{-2} at the centre of the area centralis to less than 10 mm^{-2} at the far periphery. The increase in centre size with eccentricity is

thus sufficient to offset substantially the reduction in density³¹ and suggests the existence of a regulatory process during development.

The interpretation of dendritic architecture remains a challenging problem. The issue is this. Do the branching pattern and shape of the dendritic tree have a special significance for combining the input signals to form the output train of impulses²³? Or does the form of the tree merely reflect the growth pressures and competition confronting the ganglion cell in maintaining contact with the elements delivering the input signals? Undoubtedly, the type of synaptic connections made by the ganglion cell strongly determines the message that it transmits³³. The discussions of Dowling and Boycott²⁶, Dowling³⁴ and Boycott³⁵ show how far interpretations may be taken without explicitly considering the branching patterns of the various neurones. Ganglion cells may receive input from bipolar cells both directly (dyad synapses) and indirectly (conventional synapses) through amacrine cells. In addition amacrine cells feed back on to bipolar cells (reciprocal synapses) and also make connections with other amacrine cells. The idea is that all of the rich variety of ganglion cell classes is assignable to varying proportions and types of direct and indirect inputs. The pattern of the ganglion cell dendritic tree may therefore be inconsequential for function apart from merely reflecting the spatial extent of connections.

Nevertheless, it is still possible to consider that a particular branching pattern coupled with a strategic geometric distribution of synaptic connections might be responsible for a more complex operation on the incoming excitatory and inhibitory signals than simple, weighted, linear combination. It has been argued with substantial experimental support³⁶ that inhibitory inputs on the lateral dendrite of the Mauthner cell of the goldfish have a greater attenuating effect on excitatory inputs entering far out on the dendrite than those impinging close in to the cell body.

If there is processing significance in dendritic form then the pattern of the alpha cell should be an early candidate for finer examination since its physiological counterpart, the brisk-transient class has strongly nonlinear summation properties¹⁴.

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articles

Trilobite eyes and the optics of Des Cartes and Huygens

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The thick lenses in the aggregate eyes of a group of trilobites were doublet structures designed to eliminate spherical aberration. The shape of the optically correcting interface is in accord with constructions by Des Cartes and Huygens and is dictated by a fundamental law of physics. Trilobites may have evolved such sophisticated eye-lenses to maximise optic neurone response in a dimly lit environment.

TRILOBITES, which occur in rocks ranging from Lower Cambrian (600 Myr old) to Upper Permian (250 Myr old), have the most ancient visual system known. From the beginning of their fossil record, compound eyes are present, symmetrically paired on the sides of the head. These compound eyes are primary structures and blindness in trilobites, which is not infrequent, is invariably secondary.

The primordial or holochroal eye, first found in the earliest genera, and thereafter taking many forms has numerous closely-packed lenses or prisms, each represented by a single crystal of calcite with its *c*-axis usually directed normal to the visual surface¹. Most trilobites had such eyes, the main exception being the schizochroal eye, which is confined to the Ordovician–Devonian suborder Phacopina.

Schizochroal eyes, which are the subject of this study, have large thick biconvex lenses, each separated from its neighbours by a sclera; material similar in composition and structure to the rest of the cuticle. Eyes of this kind are present in the earliest Phacopina and there is some evidence that they were derived from the eyes of holochroal ancestors by paedomorphosis.

The structure of schizochroal eyes has been described in several publications^{2,3} and it is now clear that in all known cases the lenses are doublets each of which has an upper unit of oriented calcite⁴ (with its *c*-axis normal to the visual surface) interlocking with a proximally located intralensar bowl, the composition of which has not been determined. The distinction between these two units is apparent both in specimens which are preserved in limestone and thus retain their original structure, and in those preserved in silt or mudstone and subsequently decalcified. In the former case a compositional difference between the bowl and the upper lens unit is clearly visible in thin sections or polished surfaces unless diagenetic processes have destroyed or altered the primary structure; this is usually marked by a pronounced colour change. Invariably the lenses within any one eye are identical in structure. Where specimens are decalcified, the upper lens unit disappears, but the shape of its base may be preserved on the internal mould of the fossil. This is because the bowl has become detached or has decayed shortly after the death of the trilobite, and its former space is filled with silty or sandy matrix, preserving exactly the shape of the upper unit–bowl interface. The shape of this interface is clear

in both kinds of preservation; there seem to be two basic kinds of structure with a range of intermediates between. The two fundamental kinds of lens structures were first described by Clarkson³ from Ordovician Phacopina preserved as decalcified siltstone moulds. In *Dalmanitina socialis* (Barrande) from the Caradocian (Middle Ordovician) of Bohemia, the intralensar bowl is thin and indented centrally by a small dimple (Fig. 1*a*). Really well preserved specimens (RSM Geol. 1967–32; FMNH

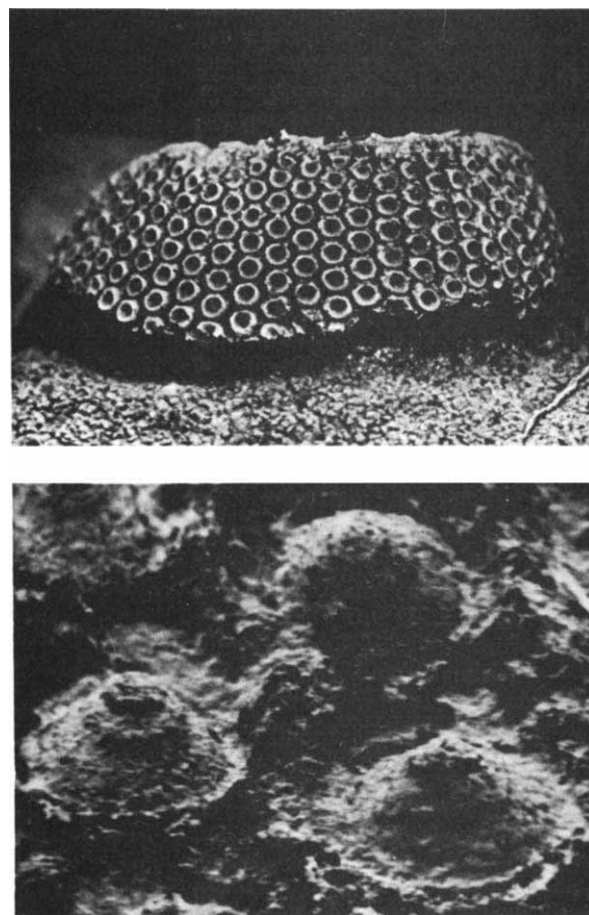


Fig. 1 *Dalmanitina socialis* (Barrande), RSM-Geol—1967–32 Letná Formation, Veselá, Bohemia, Caradocian (Ordovician). *a*, Left eye of a decalcified internal mould (steinkern) showing the intralensar bowls with central dimples. Slightly whitened with magnesium oxide ($\times 11$). *b*, Latex replica of the eye surface of the same specimen showing the central dimple of each intralensar bowl in positive relief. Scanning electron micrograph ($\times 110$).

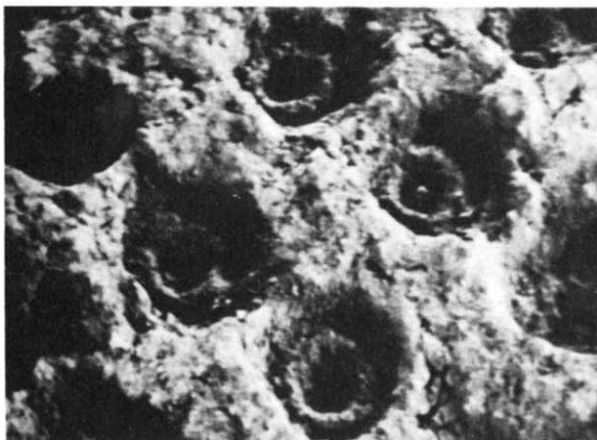
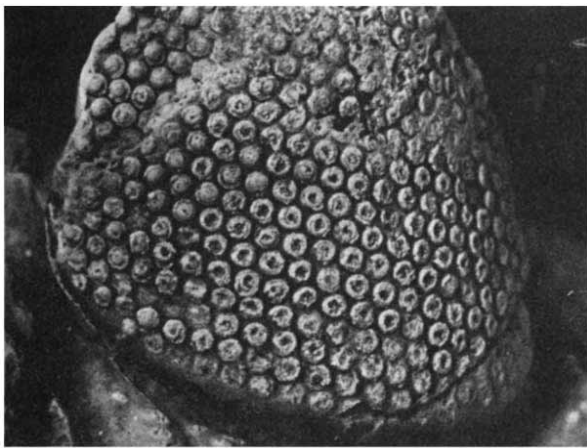


Fig. 2 *a*, *Dalmanites pratteni* Roy, FMNH P 16704, Devonian, Devil's Backbone, near Grand Tower, Jackson County, Illinois. Left eye of decalcified internal mould (steinkern) with intralensar bowls, partially filled with matrix ($\times 4$). *b*, *Zeliszkeella lapeyrei* (Bureau), Gr. I. 40192. Llandeilian. (Ordovician). Traveusot-en-Guichen near Rennes, France. Part of the left eye of a decalcified internal mould showing an intralensar bowl in each lens cavity. Scanning electron micrograph ($\times 110$).

UC-1707) are rare, but from these, latex replicas can be made showing, as an average over many lens units, the surface shape as in Fig. 1*b*. The original reconstruction of Clarkson³ has been slightly modified after a re-examination of the same material and some new material: the bowl is deeper and the upper surface is slightly more hyperbolic. Such lenses occur in other Dalmanitidae also.

The other kind of structure originally described from the species *Crozonaspis struvei* Henry⁸, from the Llandeilian (Middle Ordovician) of Brittany, is perhaps better seen in the eye of *Dalmanites pratteni* Roy⁵ from the Devonian of Illinois (Fig. 2*a*) and in *Zeliszkeella lapeyrei* (Bureau) also from the Llandeilian of Brittany (Fig. 2*b*). Here the lens is highly convex with a large and thick intralensar bowl, indented not by a small dimple, but by a wide and hemispherical depression. Lenses of this kind are not only found in these species of Dalmanitidae, but are the norm in the Siluro-Devonian family Phacopidae, especially in the genera *Phacops* and *Reedops* in which the lenses of the eye are very large, highly biconvex and relatively few in number⁶.

The example shown in Fig. 3 is a lens in horizontal section of a large-eyed Silurian *Dalmanites*. Dark field illumination shows the difference between the intralensar bowl and the upper unit. The latter has typical calcite cleavages showing it to be a single crystal (with the *c*-axis aligned along the lens axis); these stop short at the edge of the bowl. The shape of the bowl's upper surface is intermediate between the two end types mentioned above.

Corrected optics in phacopid lenses

We first discussed the possible function of schizochroal lens structure at the NATO sponsored Trilobite and Merostome Conference in Oslo in July of 1973. Subsequently, Levi-Setti recognised that the two basic patterns of the intermediate refracting surfaces in *Dalmanitina* and *Crozonaspis* approximated in shape to that of the aplanatic surfaces described respectively by Des Cartes⁷ and Huygens⁸.

Such generally aspheric refracting surfaces belong to the class of so-called Cartesian Ovals. They were originally designed to obtain lenses free of spherical aberration. The original constructions by Des Cartes and Huygens are reproduced in Fig. 4*a* and 4*c* respectively, where for comparison, the structure³ of the lenses of *Dalmanitina socialis* (Fig. 4*b*) and *Crozonaspis struvei* (Fig. 4*d*) are also shown. The similarity between the shape of the upper unit in *Dalmanitina* and the Des Cartes construction, as well as in that of the upper unit in *Crozonaspis* and the Huygens construction is strikingly apparent.

Cartesian Ovals

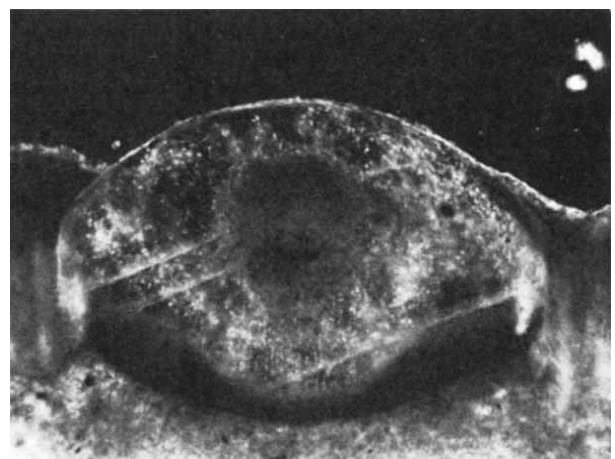
The physical principles underlying the function of the ovals of Des Cartes as optical refracting interfaces are derived from regarding these surfaces as the geometric representation of the stigmatic condition for an optical system (the requirement that a point in object space be mapped on to a point in image space). Their analytic expression, which is in general that of a fourth degree surface in Cartesian coordinates, takes a particularly significant form when cast in bipolar coordinates⁹. If the distances of a point *P* from two fixed points *O* and *O'* are called *r* and *r'* respectively, then *r* and *r'* are called bipolar coordinates of *P*. An equation connecting *r* and *r'* defining a locus for *P*, is called a bipolar equation. The ovals of Des Cartes are defined by the bipolar equation:

$$mr \pm nr' = k \quad (1)$$

where *m*, *n* and *k* are constants. If we now consider a system of two conjugate points *O* and *O'* in object (refractive index *n*₁) and image (refractive index *n*₂) space respectively, and *r* and *r'* the distances traversed by a light ray going from *O* to *O'* through a point *P* on the surface separating the two media, we can write the stigmatic condition as:

$$n_1 r + n_2 r' = c \quad (2)$$

Fig. 3 *Dalmanites* sp. Silurian. Locality unknown. Single lens in thin section in dark field illumination, showing the dark intralensar bowl, and calcite cleavages truncated by a diagenetically altered central mass. Diagenetic effects within other lenses of the same eye are very variable; in some lenses it is only incipient, whereas in others irregular masses of amorphous calcite have filled up much of the interior. This section is cut in the horizontal plane and passes through the centre of the lens ($\times 70$).



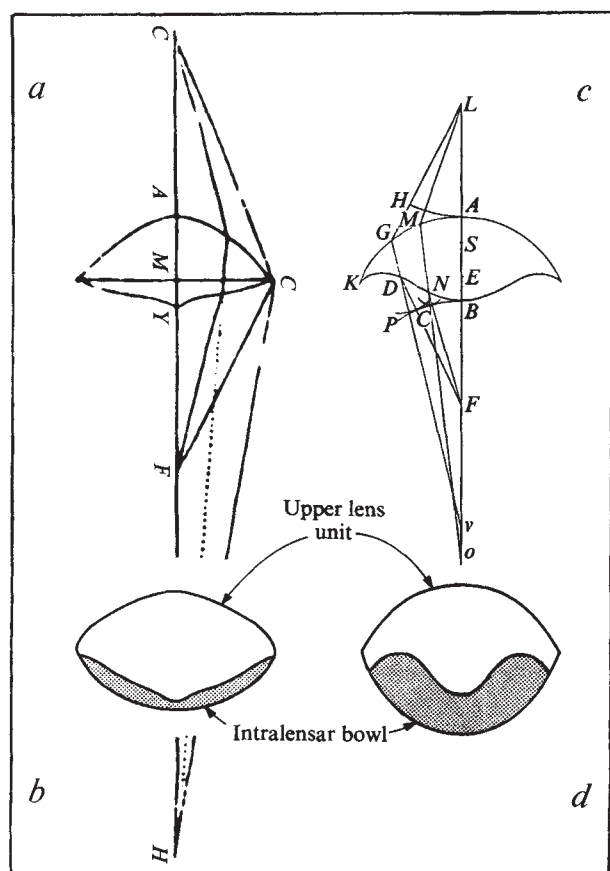


Fig. 4 a, Original construction by Des Cartes of an aplanatic lens in air, making use of two Cartesian Ovals. b, Reconstructed lens of *Dalmanitina socialis*. c, Original construction by Huygens of aplanatic lens in air making use of spherical first surface, and a Cartesian second surface. d, Reconstructed lens of *Crozonaspis struvei*³.

with c as a constant. This equation implies that whatever the position of the point P on the interface of the two media, the optical paths (represented by the left hand side of equation (2)) between the conjugate points O and O' must be the same. Alternatively, the shape of the interface must be such that light will traverse the path from O to O' in equal times, either travelling along the straight line connecting the two points (the shortest path hence the shortest time) or any other off-axis path. In his construction of a lens free of spherical aberration, Huygens⁸ adhered explicitly to this condition. Ultimately, equation (2) is a specialisation of Fermat's principle of least times, which states that no matter to what kind of reflection or refraction a ray is subjected, it travels from one point to another in such a way as to minimise the time taken. We recognise that equation (1), restricting to the $+$ sign, and equation (2) have identical forms. The bipolar representation of the ovals of Des Cartes thus acquires an immediate physical meaning through fulfilling the stigmatic condition when describing the shape of an optical interface. In this sense the ovals of Des Cartes represent the most natural economical solution to the problem of constructing optical elements free of spherical aberration. In our derivation, the only physics involved consists of Fermat's principle: Snell's (or Des Cartes') law of refraction is in fact already an implicit consequence of the former, as first demonstrated by Huygens on the basis of the wave theory of light. The ovals of Des Cartes could of course be described more laboriously with recourse only to the (weaker) refraction laws⁷.

Optical function of phacopid lens doublets

Were it not for the presence of the intralensar bowl in the trilobite lenses, there would be virtually a one-to-one correspondence in

shape and function between trilobite lenses and the optimal construction of Des Cartes and Huygens (allowing for differences arising from the fact the trilobite lenses operate in water rather than in air).

The function of the intralensar bowl has been investigated empirically by two approaches: first, graphical ray-tracing through the lens structure for example, that of *Crozonaspis*, and second, constructing a large-scale model of a *Crozonaspis* lens.

In the problem at hand we assume that the refractive indices of the media in contact with the entrance surface (seawater, $n = 1.33$) and the exit surface (body fluid, $n = 1.34$) are known. We also assume that the refractive index along the axis of the upper lens unit is equal to that along the c -axis of calcite ($n = 1.66$).

Given the interface profile actually observed, the unknowns are the refractive index of the intralensar bowl and the focal distance of the lens doublet as a whole. In principle the problem is completely determined by making use of the two constraining conditions: the stigmatic condition as an extension of equation (2); and the requirement of continuity of the light rays at each refracting surface. (It should be noted that since equation (2) satisfies Fermat's principle, the law of refraction does not represent an independent constraint.)

Using both approaches, by trial and error, we have determined that the intralensar bowl of *Crozonaspis* should have a refractive index of about 1.63, and the resulting focal length equals approximately one lens thickness measured from the exit surface. The f /number (inverse of the relative aperture) of this lens, operating in water is $\sim f/1.1$.

Given the shape of the upper unit as observed in *Crozonaspis* the intralensar bowl is thus seen as a necessary element to ensure convergence of an incident parallel beam of light to a sharp focus, when the lens is immersed in water. The intermediate Cartesian interface acts as a correcting surface, to make the doublet aplanatic.

The graphical ray tracing construction for the corrected lens versus a similar uncorrected one is shown in Fig. 5a, where the effect of spherical aberration for the latter case can be appreciated. The experimental verification of the function of the *Crozonaspis* lens is illustrated in Fig. 5b, which shows the focusing of a parallel beam of white light by a large scale model of the lens. Considering the uncertainties inherent in this reconstruction of the profile of the lens and the imperfections in the manufacture of the model, the focal plane of the corrected lens is remarkably well defined. The value of refractive index derived for the intralensar bowl may give a clue to its unknown chemical composition. Calcite was probably present, possibly together with organic material such as chitin.

Advantages and disadvantages

The fact that this remarkable lens doublet system functions in the manner empirically determined, provides independent evidence for the presence of oriented calcite⁴ in the upper unit *in vivo*. It can, moreover, easily be appreciated that such a system is adapted for light collection and for the formation of a sharp and undistorted image in its focal plane.

Calcite is the basic structural component of the trilobite exoskeleton¹⁰, but its orientation in the lenses is optimised to the function being performed. In the first place, this orientation is the direction along which the ordinary and extraordinary rays of calcite propagate with equal velocity, thus eliminating birefringence for paraxial rays. In the second place, along the c -axis the refractive index is maximal ($n = 1.66$), thus yielding the largest possible relative aperture (and thus optimising light-gathering) for a lens of the shape given.

The production of a good quality image, however, may not have been the main advantage to the trilobite of evolving such lenses. Because it is (to a first approximation) free of spherical aberration, the distribution of light intensity along the axis peaks more sharply in the region of the focal plane than it would in

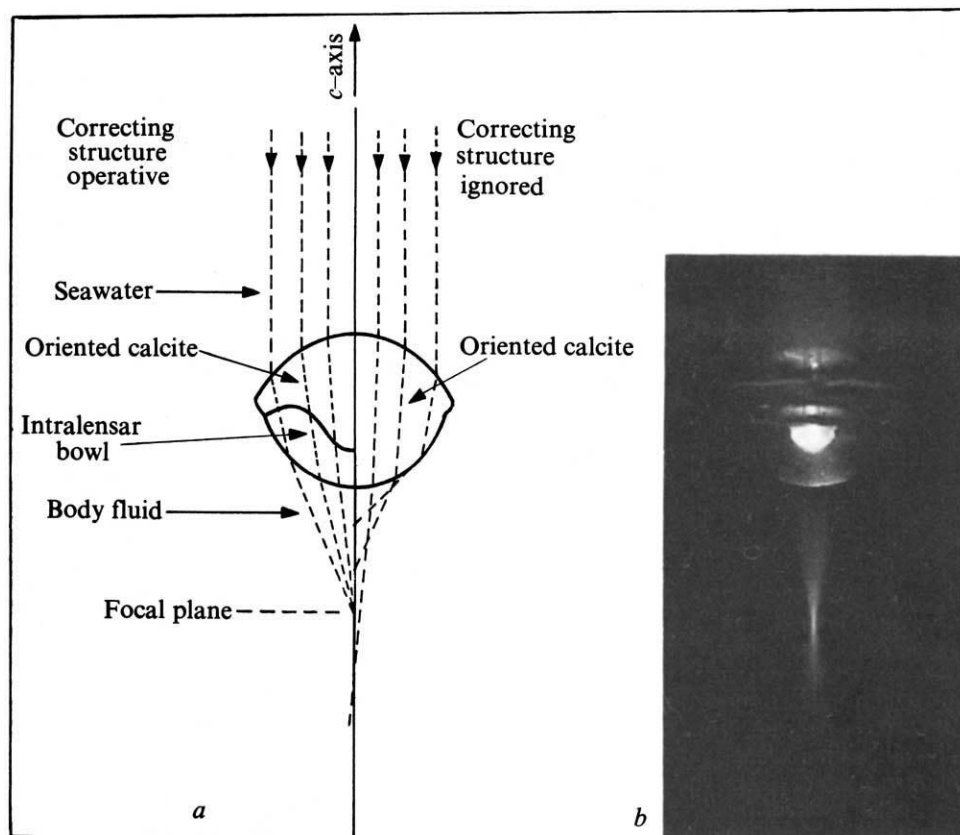


Fig. 5 *a*, Ray tracing through the lens of *Crozonaspis struvei*. On the left of the axis, the corresponding structure is assumed operative for indices of refraction: seawater 1.33, oriented calcite of upper unit 1.66, intralensar bowl 1.63, body fluid 1.34. On the right of the axis, the lens is assumed to have no internal structure, and made of oriented calcite $n = 1.66$. *b*, Parallel beam of white light incident upon a large scale model of a *Crozonaspis* lens immersed in water, where the upper unit is made of oriented calcite ($n = 1.66$), and the intralensar bowl is made of polysulphone ($n = 1.63$). The light beam is made visible by a small amount of milk mixed with the water tank. The combination of refractive indices represent an optimum condition, arrived at after testing with intralensar bowls made of various plastics.

an uncorrected lens. A measure of this improvement is the full width at half maximum of such distribution. Scaled down to the actual size of the *Crozonaspis* lens (250 μm), we estimate the above width at about 20 μm , and possibly less. If the lenses were uncorrected, the corresponding width would approximate to about 100 μm . The increased concentration of light in the focal plane could conceivably raise the level of illumination in a dimly lit environment above the neural response threshold at the retina.

The use of a doubly refracting mineral such as calcite to construct a lens may seem disadvantageous. This drawback has been shown to be minimised by the orientation of the c -axis to coincide with the optic axis of the lens. Only for an object placed considerably off-axis would a secondary (extraordinary) image be formed, at varying depths, depending on the angle of incidence, but always deeper than the ordinary (o ray) image. As a consequence, the e ray image will not coincide with that formed by the o ray, nor will it focus in the same plane. One could envisage several means by which this ghost image could have been eliminated, although in any case it may have been of little consequence. A retina of limited thickness located in the image plane of the o ray would automatically filter out the e ray image: although accommodation of the eye would require a movable retina, a remarkable depth of field would still be available even with a fixed one. From the optical parameters of phacopid lenses, and requiring a circle of confusion in the retinal plane of diameter as small as 1 μm (the resolution limit due to diffraction), the depth of field would extend from several mm (~ 1 trilobite length) to infinity. Alternatively, one could invoke the use of photoreceptors sensitive only to the direction of polarisation of the o ray, thus eliminating the effect of the e ray image (the polarisation analysing capability of the rhabdomes is well known).

Finally, one could object that chromatic aberration may have offset the correction of spherical aberration. This is unlikely, however, since the environment under the sea is essentially monochromatic even at moderate depths.

Visual function of schizochroal eye

The function of schizochroal eyes can be better appreciated when contrasted with that of holochroal eyes. Holochroal eyes in trilobites normally have pronounced curvature in both the azimuthal and vertical planes (often assuming a toroidal shape) and have numerous optical elements which could, as in insect and crustacean eyes, have formed a true visual mosaic. Schizochroal eyes, however, have too few optical units to form a detailed mosaic image. Furthermore, the arrangement of the lenses in files often widely separated and pointing in diverging directions, leads to a discontinuous coverage of the object space². Such eyes as these were formerly thought² to be capable only of gross movement perception, though the discovery of corrected lenses now calls this view into question.

What use did phacopid trilobites actually make of their corrected lenses? They may have been used primarily as light concentrators, as has recently been proposed¹¹ for *Limulus polyphemus*. Alternatively, they may have served a more complex function. If the photoreceptor had the form of a thin retina, as suggested by the aplanatic properties of the lens, and if it was composed of numerous subunits, then the corrected image formed by the lens at the level of the focal plane or immediately beneath it, could be perceived as a micromosaic. The schizochroal eye could then be regarded more as an aggregate of individual eyes, each surveying a different part of the object space, rather than as a true compound eye. Evolution would have in this case proceeded from the external mosaic scheme of the ordinary compound eye towards the utilisation of an internal mosaic, much as in the lens-retina eye system of more advanced life forms. It is unfortunate that the genetic information of such a perfected visual apparatus became lost to further evolution in the animal kingdom, when the phacopid trilobites became extinct.

The presence of crystallised *in vivo* calcite in the lenses of fossilised trilobite specimens may prove valuable in a different context. It is conceivable in fact, that this primary mineral retained its original isotopic composition, in equilibrium with

the depositional environment. If so, trilobite lenses may afford an unprecedented opportunity for determining the temperatures of Palaeozoic seas using the $^{18}\text{O}/^{16}\text{O}$ relative abundance method¹².

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Evolutionary implications of different types of microbial enzymology for L-tyrosine biosynthesis

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Several patterns of enzymology for L-tyrosine biosynthesis exist in modern microorganisms, each differing in the apparent degree of regulatory efficiency. The extent of pathway evolution in a particular organism may reflect the relative selective pressure for regulation encountered in different ecological niches.

THE biochemical similarities of major metabolic pathways utilised in nature have reinforced a long standing concept of the biochemical unity of all forms of life^{1,2}. Against this background, instances of diversity offer intriguing prospects for insight into evolutionary relationships. Biosynthetic pathways for amino acids are undoubtedly ancient and generally exemplify the concept of biochemical unity. The occasional existence of different amino acid-forming pathways seems to reflect significant phylogenetic divergencies, for example, biosynthetic pathways for L-lysine³, L-methionine^{4,5} and L-tyrosine^{6,7}.

Several enzyme arrangements that serve L-tyrosine biosynthesis in various microorganisms differ in pattern of regulatory control. At one extreme, species of blue-green bacteria (algae) possess a seemingly primitive pathway (pretyrosine) which has a less refined regulation, in contrast with that of the 4-hydroxyphenylpyruvate pathway of *Escherichia coli*. We report here results obtained with several other microorganisms in which features of pathway enzymology and regulation seem intermediate between those of blue-green bacteria and enteric bacteria. These data, considered together with previous published results, suggest an overall picture of pathway evolution in which modern organisms possess pathways representing different stages of an apparent evolutionary sequence. We propose that the selective pressure for regulation of L-tyrosine biosynthesis varies substantially within the ecological niche occupied by a given microbial group, and that the evolutionary stage of pathway development seen in modern organisms reflects the degree of selective pressure for regulation.

Pathways of L-tyrosine biosynthesis

The biosynthetic pathway for aromatic amino acids begins with the condensation of erythrose-4-phosphate and phosphoenolpyruvate, the first of seven enzyme reactions that culminate

with the formation of chorismate⁸. Chorismate is either used for tryptophan synthesis or converted to prephenate. So far as is known, all organisms able to synthesise L-tyrosine and L-phenylalanine utilise prephenate as the last common intermediate of a branching pathway. Although the enzyme sequence of the biosynthetic branchlet leading to L-phenylalanine may be universal, different L-tyrosine pathways exist. The pretyrosine and 4-hydroxyphenylpyruvate pathways, named for the intermediate between prephenate and L-tyrosine, are illustrated in Fig. 1. In each case both transamination and decarboxylation-dehydrogenation reactions occur—but in reverse order. The enzymes of each pathway are quite specific in organisms such as *Bacillus subtilis* and *Agmenellum quadruplicatum*^{6,7}, the most studied species exemplifying the 4-hydroxyphenylpyruvate and pretyrosine pathways, respectively. Hence, in *B. subtilis* a partially purified dehydrogenase utilises prephenate (prephenate dehydrogenase) but is not reactive with pretyrosine. No other dehydrogenase able to utilise pretyrosine was found in crude extracts or in fractions obtained during purification procedures. An aromatic transaminase of *B. subtilis* that was reactive with phenylpyruvate did not utilise prephenate⁷. Attempts to resolve other transaminases that might be reactive with prephenate were unsuccessful.

In *A. quadruplicatum* a single dehydrogenase is specific for pretyrosine, and no prephenate dehydrogenase activity was detected^{6,7}. A single transaminase would seem to catalyse the transamination step within each pathway branchlet (that is, prephenate transaminase and phenylpyruvate transaminase). Transamination occurs far better with prephenate or phenylpyruvate than with 4-hydroxyphenylpyruvate⁷; likewise, in the non-biosynthetic direction L-phenylalanine is a far better amino donor than is L-tyrosine⁸. So far, the exclusive presence of enzymes of the pretyrosine pathway are known only in blue-green bacteria. The 4-hydroxyphenylpyruvate pathway for L-tyrosine synthesis seems to be the most widely distributed (Table 1), based on the comparative survey of dehydrogenase specificities in a limited range of microorganisms⁷.

In addition to the pretyrosine and 4-hydroxyphenylpyruvate pathways, a third type of enzymology exists (denoted ambiguous in Table 1). Here transaminase and/or dehydrogenase activities are markedly ambiguous in substrate recognition. For example, *Pseudomonas aeruginosa* has the enzymological potential to synthesise L-tyrosine by either pathway. Table 2 shows the

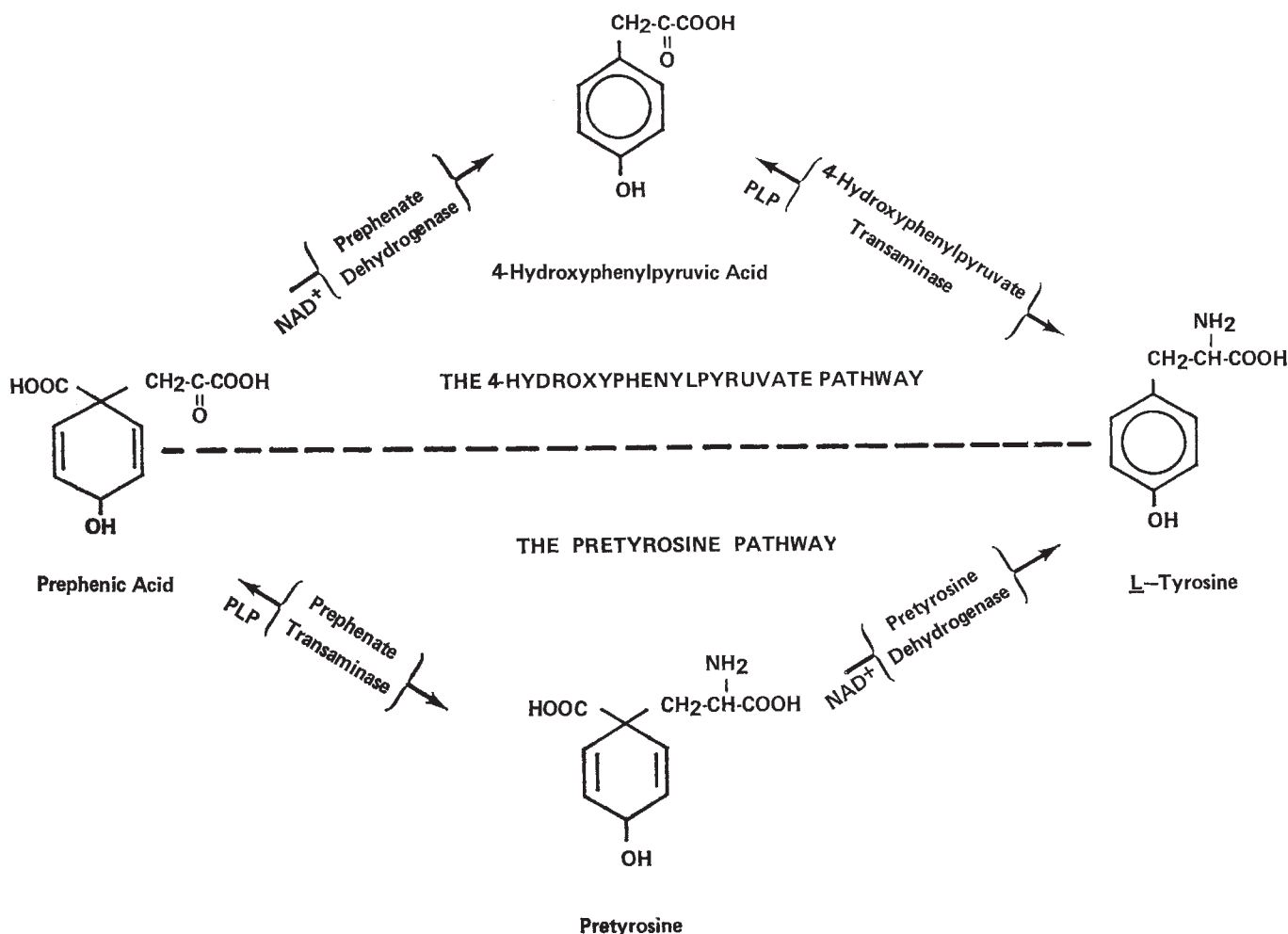


Fig. 1 Enzyme sequences of L-tyrosine biosynthesis. The upper sequence is the 4-hydroxyphenylpyruvate pathway, and the lower sequence is the pretyrosine pathway. A third ambiguous type refers to the presence of ambiguous transaminase (prephenate/4-hydroxyphenylpyruvate) and dehydrogenase (prephenate/pretyrosine) activities that allow either sequence to operate. NAD⁺, nicotinamide adenine dinucleotide; PLP, pyridoxal-5'-phosphate.

substrate ambiguities observed for enzymes of *Neurospora crassa* and *P. aeruginosa*. In *N. crassa* only prephenate-specific dehydrogenase activity was found. A nonspecific transaminase, however, utilises either prephenate or 4-hydroxyphenylpyruvate. We have found (unpublished observations with S. L. Stenmark-Cox) that transaminase reactivity with prephenate in *N. crassa* can be expressed *in vivo*, resulting in dead-end accumulation of pretyrosine. Prephenate dehydratase mutants of *N. crassa* accumulate prephenate which is readily transaminated to pretyrosine, and substantial amounts of pretyrosine are accumulated in the growth medium. In *P. aeruginosa* an apparent single species of dehydrogenase is recovered⁹ that reacts with either prephenate or pretyrosine. Although several transaminase activities were resolved from crude extracts of *P. aeruginosa* (I, II and III of Table 2), each transaminase species exhibited similar substrate ambiguity with respect to prephenate and 4-hydroxyphenylpyruvate utilisation.

found to be feedback inhibited efficiently by L-phenylalanine (Fig. 2, bottom row).

In *B. subtilis* (Fig. 2, right panel), the tyrosine and phenylalanine sequences are symmetrically analogous, each containing an initial irreversible reaction followed by a transamination step.

Table 1 Pathways of L-tyrosine biosynthesis

Taxonomic occurrence	Type of enzymology	Apparent efficiency of regulation
Blue-green bacteria	Pretyrosine	+
<i>Pseudomonas</i>	Ambiguous [D], [T]	++
<i>Neurospora</i>	Ambiguous [T]	
<i>Aerobacter</i>		
<i>Bacillus</i>		
<i>Brevibacterium</i>		
<i>Clostridium</i>	4-Hydroxyphenylpyruvate	+++
<i>Escherichia</i>		
<i>Serratia</i>		
<i>Phaseolus</i> *		
<i>Saccharomyces</i>		

Data supporting this summary are given or cited in refs 6 and 7. The most detailed published data to document *Pseudomonas* and *Neurospora* enzymology patterns are given in Table 2. Details of pretyrosine enzymology in *A. quadruplicatum* and references documenting the distribution of pretyrosine, 4-hydroxyphenylpyruvate or ambiguous pathways in nature are given in refs 6 and 7. [D], [T] Means that both dehydrogenase and transaminase activities exhibit substrate ambiguities, whereas [T] indicates that only transaminase (prephenate/4-hydroxyphenylpyruvate) is ambiguous in substrate recognition. These pathway types are illustrated in Fig. 4.

* Our recent results with cotyledon tissues from species of *Phaseolus* reveal very substantial pretyrosine pathway enzyme activities.

Regulation of three pathway types

Three general patterns of L-tyrosine biosynthesis are discernable, exemplified by *A. quadruplicatum* (pretyrosine), *P. aeruginosa* (ambiguous) and *B. subtilis* (4-hydroxyphenylpyruvate). Each organism possesses a distinctive pattern of regulation for L-tyrosine synthesis, as compared schematically in Fig. 2. Unlike the L-tyrosine branchlet, the enzyme sequence within the L-phenylalanine branchlet is invariant in all species studied (that is, prephenate dehydratase followed by phenylpyruvate transaminase). Inevitably, prephenate dehydratase has been

Table 2 Multi-substrate reactivities of L-tyrosine biosynthetic enzymes

Microorganism	Dehydrogenase substrates		(nmol min ⁻¹ mg ⁻¹)	Transaminase substrates	
	Prephenate	Pretyrosine		Prephenate	4-Hydroxyphenylpyruvate
<i>N. crassa</i>	8.2	0		6.4	10.3
<i>P. aeruginosa</i>	24.6	32.4	I	32.9	131.8
			II	56.9	89.8
			III	124.8	274.8

Mycelia of a wild-type strain of *N. crassa* (74-OR 23-1A) were collected after 72 h growth at 25 °C in Vogel's minimal medium²³ supplemented with 2% (w/v) sucrose. Cultures were started from heavy conidial inoculations. Mycelial yields from 10 l of medium were lyophilised. Extracts were prepared by addition of mycelial powder to 50 mM potassium phosphate buffer, pH 7.5, containing 50 mM KCl, 2 mM MgCl₂ and 20% (w/v) sucrose. The suspension was stirred at 4 °C for 20 min and centrifuged at 25,000g for 30 min. The clarified supernatant was dialysed at 4 °C against 500 volumes of buffer overnight. This cell-free extract was used for dehydrogenase and transaminase assays. In *P. aeruginosa* partially purified enzyme preparations were used. The dehydrogenase and transaminase I were recovered from DEAE cellulose as before⁹. Transaminase II and III are the leading and trailing bands of activity eluted from hydroxylapatite⁹. Details of the dehydrogenase assays were previously given^{9,10}. The validity of NADH formation as a measure of pretyrosine dehydrogenase activity was verified by the fluorometric measurement of tyrosine²⁴. The following reaction mixture was used to measure transaminase activities: 50 µl of 40 mM glutamate, 100 µl of 5 mM prephenate or 4-hydroxyphenylpyruvate, and 50 µl of enzyme preparation. After 20 min of reaction at 37 °C, the volume of the reaction mixture was brought to 5 ml with 2.5 N NaOH. When prephenate was used as substrate, 100 µl of 1 N HCl was added and the incubation was continued for 10 min prior to the addition of NaOH to convert prephenate to phenylpyruvate. Phenylpyruvate absorbance was measured at 320 nm and 4-hydroxyphenylpyruvate absorbance was measured at 331 nm, using a Gilford spectrophotometer.

Regulation of each pathway is classically straightforward. Excess L-tyrosine feedback inhibits prephenate dehydrogenase (Fig. 2, top right), while excess L-phenylalanine feedback inhibits prephenate dehydratase (bottom right). In *E. coli* the pathway and its regulation is essentially identical to that of *B. subtilis* with the exception that prephenate dehydratase and prephenate dehydrogenase are both multifunctional proteins, each also catalysing the chorismate mutase reaction¹¹.

Unlike *B. subtilis*, the pretyrosine pathway of *A. quadruplicatum* contains an initial reversible step, activity of which is not affected by L-tyrosine. A degree of control is indirectly accomplished through the activating effect of L-tyrosine on prephenate dehydratase⁶. Thus, high intracellular levels of L-tyrosine would tend to favour flow of metabolites towards L-phenylalanine and away from L-tyrosine (Fig. 2, top left). The apparent *K_m* for prephenate of the competing prephenate dehydratase (0.23 mM) and prephenate transaminase (1.05 mM) activities suggest that flow towards phenylalanine is intrinsically favoured. The transaminations of phenylpyruvate and prephenate are probably catalysed by a single protein⁶; this has the same affinity (*K_m*, 1.05 mM) for prephenate or phenylpyruvate but has a five-times greater *V_{max}* with phenylpyruvate. The conditions assumed at the top left of Fig. 2 (excess tyrosine, limiting phenylalanine) would promote a relative increase in the intracellular supply of phenylpyruvate together with a decreased supply of prephenate (owing to enhanced prephenate dehydratase activity). This would favour transaminase function as phenylpyruvate transaminase (rather than prephenate transaminase). On the other hand, high intracellular levels of L-phenylalanine would tend to shift prephenate flow towards L-tyrosine formation (Fig. 2, bottom left). Inhibition of prephenate dehydratase would increase levels of prephenate substrate for transamination while decreasing levels of phenylpyruvate substrate for transamination.

The regulatory pattern for L-tyrosine biosynthesis in *P. aeruginosa* can be viewed as intermediate between those of *A. quadruplicatum* and *B. subtilis*. To whatever extent the pretyrosine reaction sequence may be realised *in vivo* owing to substrate ambiguity in *P. aeruginosa*, the overall picture of regulation (or lack of it) would resemble that of *A. quadruplicatum*. The middle panel of Fig. 2, however, depicts the probable domination *in vivo* of the 4-hydroxyphenylpyruvate sequence in *P. aeruginosa*⁷. Prephenate is preferentially channelled towards L-phenylalanine by virtue of a single, multi-functional protein¹² which catalyses both the chorismate mutase and prephenate dehydratase reactions. Prephenate dehydrogenase, which is subject to relatively weak competitive inhibition by L-tyrosine, has been characterised recently⁹. When L-phenylalanine is present in excess (Fig. 2, bottom middle) inhibition of the second half-reaction of the enzyme complex leaves the prephenate formed in the first reaction free for utilisation by

prephenate dehydrogenase¹². The pseudomonad pattern of regulation resembles *B. subtilis* in its feedback regulation of the initial reaction of the tyrosine branchlet. On the other hand, the pseudomonad enzymological pattern resembles that of blue-green bacteria in the preferential flow of chorismate toward L-phenylalanine and in the dominating regulatory role of L-phenylalanine, directly in the phenylalanine branchlet and indirectly in the tyrosine branchlet¹². Preferential entry of prephenate into the L-phenylalanine branchlet has been observed in several other microorganisms^{13,14}.

Possible ancestral pathway

It is reasonable to assume that the most ancient biochemical pathways were unregulated, for regulatory properties seem to be secondary refinements that must have followed the primary acquisition of catalytic capabilities. Branching amino acid pathways such as those forming aromatic amino acids and branched-chain amino acids probably originated as simple, uncomplicated pathways forming a single amino acid. Subsequent additions of pathway branchlets resulted in families of related amino acids. Certain abiotically-formed amino acids in the primitive *milieu* inevitably became growth-limiting before others. Biosynthesis of a limiting aromatic amino acid would have conferred a selective advantage on organisms with this capability. If one assumes that the tryptophan sequence (or any other chorismate-containing pathway, for example, to folate or ubiquinone) originated before the phenylalanine and tyrosine pathways, tyrosine and phenylalanine-forming enzymes may have evolved as shown in Fig. 3, a sequence which is based on non-enzymatic chemical reactivities of chorismate and prephenate. Tiny amounts of L-phenylalanine and pretyrosine could have been synthesised from chorismate in ancient cells without the benefit of new enzymes. Chorismate is readily rearranged thermally to form prephenate¹⁵, and this conversion can be substantial at physiological temperatures. Prephenate, in turn, is quite labile at acid pH, and even mildly acidic conditions consistent with cell growth can promote acid-catalysed conversion to phenylpyruvate¹⁶. Assuming that some ancient transaminase possessed the broad specificity that commonly characterises modern transaminase enzymes, the dual possibility existed for transamination of phenylpyruvate to L-phenylalanine and/or of prephenate to pretyrosine. Like prephenate, pretyrosine is acid-labile⁶ and would readily be converted to L-phenylalanine.

Subsequent selective advantages arising from the biosynthesis of L-phenylalanine would favour the evolutionary development of enzymes to catalyse the previously non-enzymatic steps more efficiently. Pretyrosine may also have been a component of ancient proteins, and indeed the possibility that it may be an amino acid residue in modern proteins of blue-green bacteria has not yet been explored. In any event, the availability of the

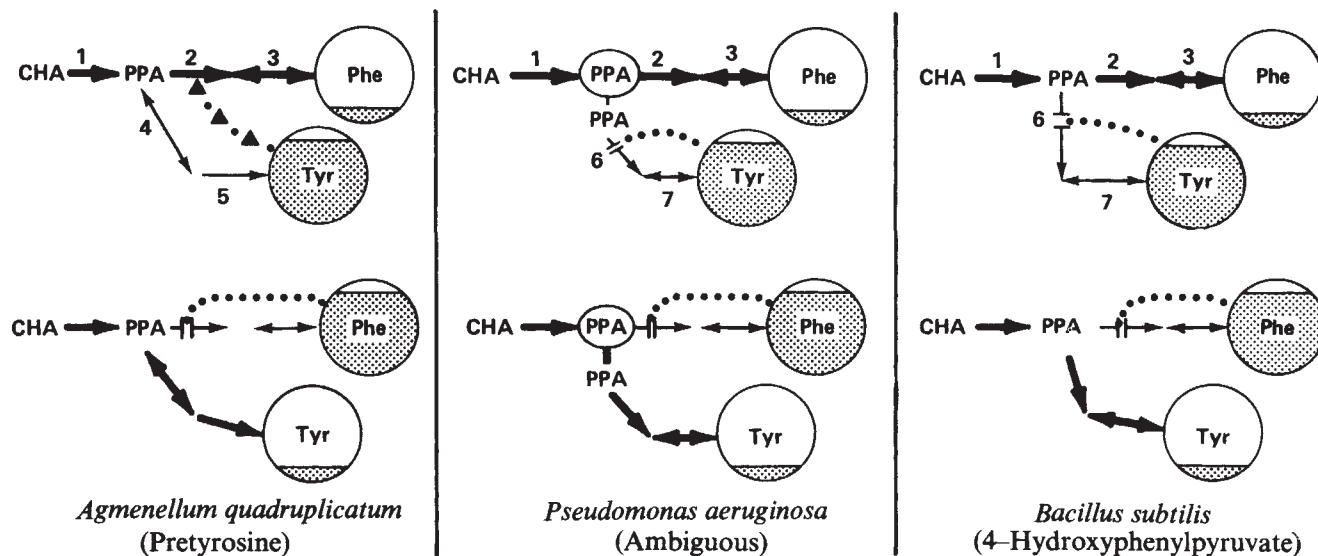


Fig. 2 Regulatory patterns of L-tyrosine/L-phenylalanine biosynthesis in *A. quadruplicatum* (left), *P. aeruginosa* (middle), and *B. subtilis* (right). The upper row of diagrams depicts an unbalanced nutritional state where L-tyrosine (Tyr) is available in excess while L-phenylalanine (Phe) is limiting. At the other extreme (bottom row) L-phenylalanine is present in excess while L-tyrosine is limiting. The flow pattern of intermediary metabolites is indicated by heavy arrows. CHA, chorismate; PPA, prephenate. In the middle panels the circled PPA denotes PPA that is channelled by the 1-2 enzyme complex¹², and which is not readily available to enzyme 6. —•••, feedback inhibition; ▲•▲, enzyme activation. Enzyme notations: 1, chorismate mutase; 2, prephenate dehydratase; 3, phenylpyruvate transaminase; 4, prephenate transaminase; 5, pretyrosine dehydrogenase; 6, prephenate dehydrogenase; 7, 4-hydroxyphenylpyruvate transaminase. References documenting the regulatory effects shown have been detailed elsewhere for *A. quadruplicatum*⁶, *P. aeruginosa*^{9,12} and *B. subtilis*^{10,27-29}.

pretyrosine molecule would permit the development of L-tyrosine-synthesising capability following acquisition of a single new enzyme. Presumably, this occurred by duplication of a gene encoding an ancient dehydrogenase, followed by mutational modification of that duplicated gene^{17,18} to produce a pretyrosine-reactive dehydrogenase. The hypothesis that L-phenylalanine biosynthesis preceded L-tyrosine biosynthesis is consistent with the uniformity of L-phenylalanine enzymology and regulation in nature (Fig. 2).

Evolutionary alteration of specificities

The ultimate ancestral pathway for L-tyrosine biosynthesis that is hypothesised in Fig. 3 is identical to that of modern blue-green bacteria. In Fig. 4, blue-green bacteria are depicted as having the most primitive pathway while *E. coli* possesses one of the most evolved pathways of L-tyrosine biosynthesis. Assuming that the 4-hydroxyphenylpyruvate pathway of modern *E. coli* originated as a pretyrosine sequence, one can view the L-tyrosine pathways of other modern organisms as reflecting stages of pathway evolution that may once have been intermediate evolutionary stages in *E. coli*.

Beginning with the transaminase and dehydrogenase specificities found in modern blue-green bacteria, transaminase and dehydrogenase enzymes may have evolved through stages similar to those still retained in modern pseudomonads and *Neurospora*, finally developing the reversed specificity of enzymes found in the 4-hydroxyphenylpyruvate pathway of *E. coli*. Thus, in the stage exemplified by *Pseudomonas*, a single NAD-dependent dehydrogenase has evolved reactivity with prephenate and diminished reactivity with pretyrosine. Likewise, a single transaminase possesses an increased reactivity with 4-hydroxyphenylpyruvate but less reactivity with prephenate. Continued selection for this flip-flop change of specificity, resulting in loss of pretyrosine pathway capability and increased 4-hydroxyphenylpyruvate pathway capability, is shown in Fig. 4 (lower right). The pathway of *N. crassa* is shown as intermediate between pseudomonads and *E. coli*, owing to the lack of ambiguity of dehydrogenase (as in *E. coli*) but the

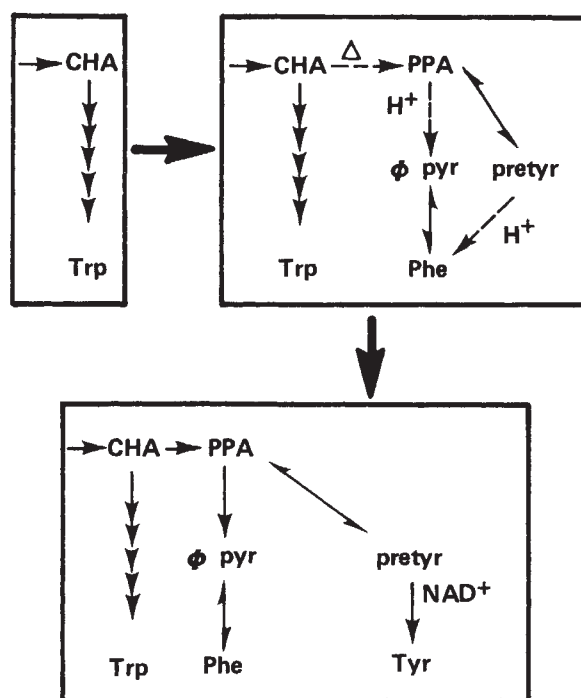


Fig. 3 Hypothetical evolution of capability for L-tyrosine and L-phenylalanine biosynthesis in ancestral cells. Enzymatic capability for synthesis of the aromatic amino acid family is postulated to have evolved in the order: L-tryptophan, L-phenylalanine and L-tyrosine. CHA, chorismate; PPA, prephenate; φ pyr, phenylpyruvate; pretyr, pretyrosine; Trp, L-tryptophan; Phe, L-phenylalanine; Tyr, L-tyrosine. Solid arrows and dotted arrows represent enzymatic and non-enzymatic reactions, respectively. Prephenate and pretyrosine are acid-labile, undergoing quantitative conversion to phenylpyruvate and L-phenylalanine respectively. Chorismate is converted to prephenate at elevated temperature (Δ).

presence of prephenate/4-hydroxyphenylpyruvate transaminase (as in pseudomonads). Finally, *E. coli* exhibits dehydrogenase and transaminase specificities that are distinct from those seen in blue-green bacteria.

Pathway regulation and ecological niche

The 4-hydroxyphenylpyruvate pathway of *B. subtilis* and many other eubacteria such as *E. coli* seems to be more sophisticated (in terms of regulatory versatility) than the other L-tyrosine-forming pathways of various microorganisms. This is not to suggest that *E. coli*, for example, is biologically superior to other microorganisms in any general sense. The presence of a more highly evolved pathway implies an evolutionary response to strong selective pressures. It is clear that biological success in one ecological niche involves different selective pressures than in another. We suggest that the regulatory efficiency of each microbial type of enzyme sequence for L-tyrosine synthesis is generally appropriate to the ecological niche in which a given microbe is successful. At one extreme, *E. coli* resides in the gut of man where the nutritional regimen is one of periodic feast and famine. A selective advantage results from a simple, direct and efficient regulation of both enzyme activity and synthesis. *E. coli* is geared for substantial amino acid biosynthesis during famine as well as for efficient utilisation of exogenous amino acids during feast.

The ecological relationships of blue-green bacteria are quite different. The competitive advantage of blue-green bacteria in nature is their photoautotrophic mode of metabolism. They utilise small organic molecules poorly, and heterotrophic capabilities are generally undeveloped. In fact, amino acid molecules and other organic compounds are often inhibitory to the growth of autotrophic bacteria¹⁹⁻²¹. In the ecological niche of blue-green bacteria, characterised by the constantly diluting effect of the fresh or marine water *milieu*, most L-tyrosine molecules probably originate from endogenous metabolism. When exogenous tyrosine is present under laboratory conditions, the regulation seems poor since no direct mechanism exists to decrease L-tyrosine biosynthesis. The regulatory scheme shown in Fig. 2 may, however, satisfactorily partition the flow of endogenous prephenate into the tyrosine and phenylalanine branchlets. If so, one must assume that the kinetic properties of the competing enzymes at the branchpoint are appropriate for some endogenous and relatively constant level of prephenate. Thus, the primitive stage of development of the L-tyrosine pathway may be adequate for the ecological niche of blue-green bacteria. The persistence of the pretyrosine pathway in modern blue-green organisms may reflect the lack of selective pressure for more elaborate regulation.

Enteric and pseudomonad microorganisms provide an especially apt comparison of the relationship of metabolic specialisation and regulation. The most striking metabolic capability of pseudomonad organisms is their versatile utilisation of organic compounds as sole sources of carbon, nitrogen and energy. Enteric bacteria provide the most dramatic examples of repression control of biosynthetic enzyme levels, although scarcely any repression control of biosynthetic enzymes is known in pseudomonads. On the other hand, pseudomonads provide the most impressive examples of induction control for numerous degradative enzymes. Thus, metabolism seems to be geared to biosynthesis in enteric bacteria and to catabolism in pseudomonads. Since survival value is undoubtedly equated with these metabolic capabilities, selection for the most finely tuned regulatory mechanisms may be maximised within the pathways providing metabolic specialisation. The presence of enteric-like repression control mechanisms for biosynthetic enzymes in pseudomonads would not necessarily be an advantage, since that might impose restrictions on overall capability for regulatory variation of degradative enzymes. In natural soil and water environments, substantial accumulation of free amino acids does not occur²². Pseudomonads in nature do not experience the regular exposure to high levels of amino acids and so on which place a premium on responsiveness to end-product

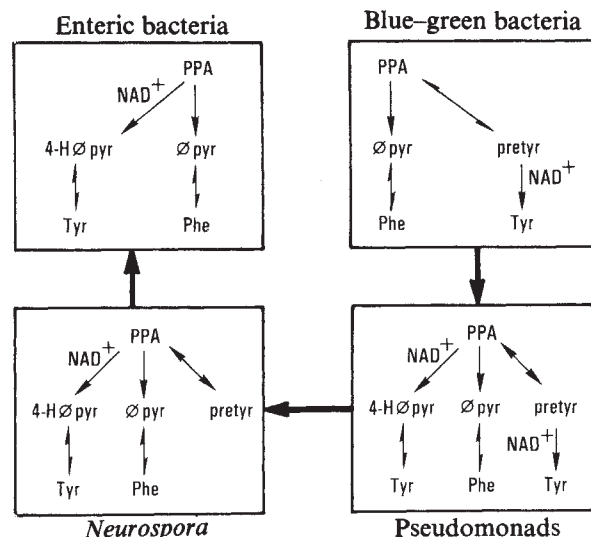


Fig. 4 The evolutionary development of the 4-hydroxyphenylpyruvate (4-Hppyr) pathway viewing the existing pathway in *E. coli* as the most highly evolved and that in blue-green bacteria (algae) as the least evolved. Abbreviations are as given in Fig. 3.

levels in enteric bacteria. Even plant or animal pathogens (such as *P. aeruginosa*) do not encounter the regularity of concentration flux, as is the case with enteric organisms. It seems reasonable that selection for efficiency of regulation would be maximised for biosynthetic pathways in enteric microorganisms. Presumably other organisms have different metabolic priorities that are dictated by differing selective pressures. For example, rigorous selection for regulatory efficiency would be expected for catabolic enzymes of pseudomonads and for enzymes of autotrophic metabolism in blue-green bacteria.

It is interesting that the apparent differences in regulatory refinement for enzymes of L-tyrosine synthesis are paralleled by the same qualitative differences in apparent regulatory efficiency for 3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase in *A. quadruplicatum*²¹, *P. aeruginosa*²⁵ and *B. subtilis* (or *E. coli*)²⁶.

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Vertical crustal movements of the Andes in Peru

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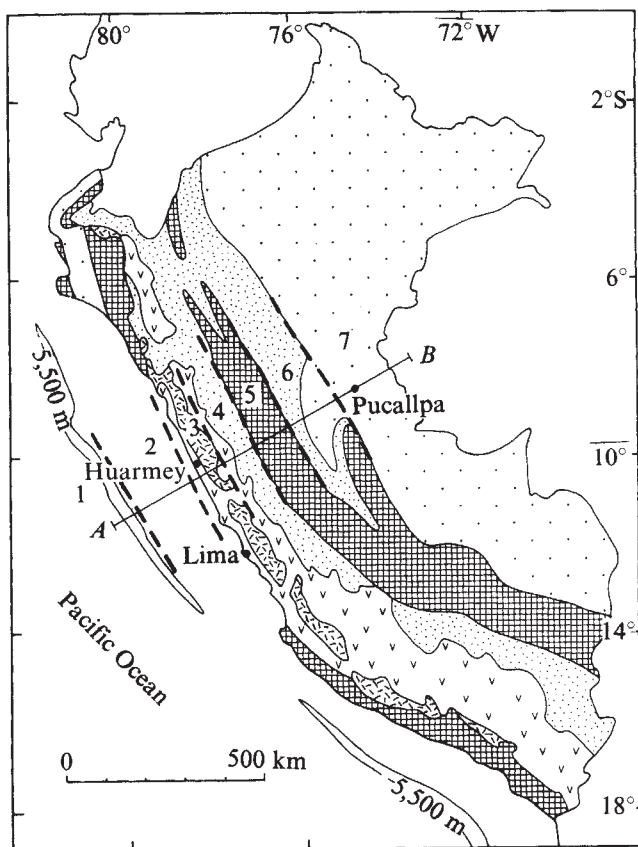
Although the Peruvian Andes provide no evidence of the widespread, strong deformation which might be expected in a tectonic belt widely held to overlie a zone of oceanic subduction, consideration of the tectonic and magmatic history shows that the occurrence of large scale, vertical block faulting in that region is not incompatible with a plate tectonic model.

The important association between vertical crustal movements and magmatism during the evolution of the Andean mountain range was recognised^{1,2} in the nineteenth century. Most mountain belts later became interpreted in the light of alpine tectonics³ and comparisons with the Alps continued throughout the period during which mountain belts were viewed as expressions of the final phase of geosynclinal cycles. The concept of plate tectonics eventually emphasised the fundamental differences between many mountain belts⁴, but the Andes was equated⁴ with the cordilleras of western North America where it was postulated that major doming of the continental margin above underthrust oceanic lithosphere has caused overthrusts on the

continent and the spread of sediments over the adjacent oceanic crust. There is little supporting evidence for this hypothesis in the Peruvian Andes^{5,6}, where consideration of the past 100 Myr of tectonic and magmatic history suggests a tectonic model which is different from that postulated⁴ for western North America. The description is based on detailed mapping of the western Andes between latitudes 10° and 10° 30' S (ref. 7) and published descriptions of adjacent regions⁸⁻¹⁰, supplemented by radiometric age determinations (P. A. Wilson, unpublished).

Regional tectonics

During Cretaceous time, northern Peru consisted of two ribbon-like belts of subsidence known as the West and East Peruvian troughs^{8,11} (Fig. 1). They were separated by a belt of relative uplift, the Marañón Geanticline⁸, and a second belt of relative uplift, the Paracas Geanticline¹², formed the western edge of the continent. Each belt was founded on a block of Precambrian basement with thin Phanerozoic cover¹³, and was bounded by



- Cainozoic sediments on Precambrian basement with some Palaeozoic and Mesozoic cover
- ▨ Cretaceous-Tertiary Coastal Batholith
- ▤ Cretaceous-Tertiary volcanics
- ▧ Mesozoic shelf sediments
- ▩ Precambrian basement and Palaeozoic cover

Fig. 1 Simplified geological map of Peru (after ref. 13) showing the tectonic framework of northern Peru during Cretaceous and early Tertiary time. 1, Oceanic crust; 2, Paracas Geanticline; 3 and 4, West Peruvian Trough (3, Paramonga Block; 4, Chavin Block); 5, Marañón Geanticline; 6, East Peruvian Trough; 7, Brazilian Shield. A-B, line of sections of Figs 2, 3 and 5.

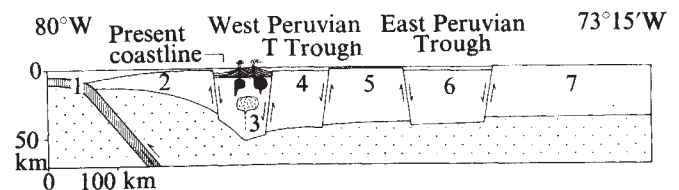


Fig. 2 Schematic section (A-B, Fig. 1) across Peru 100 Myr ago, showing subduction of oceanic crust (1), and the movement of major blocks of continental crust: 2, Paracas Block; 3, Paramonga Block; 4, Chavin Block; 5, Marañón Block; 6, East Peruvian Trough block; 7, Brazilian Shield. Casma Volcanics were erupted on the Paramonga Block from the rising Coastal Batholith (Patap Complex—black; tonalite—irregular dashes; continental crust—white; mantle—stippled; T, Tapacocha Axis.

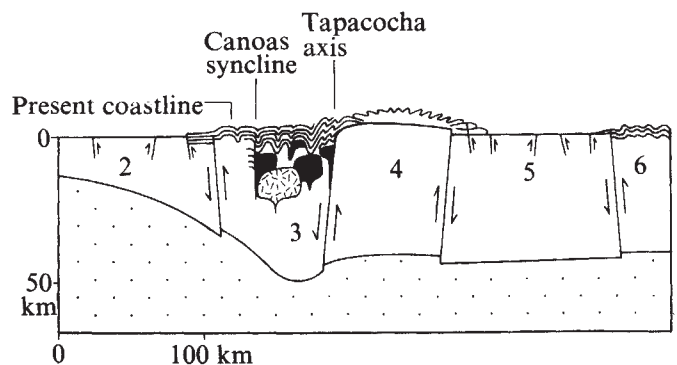


Fig. 3 Schematic section (part of A-B, Fig. 1) showing the different kinds of deformation of Mesozoic sedimentary and volcanic rocks which resulted from uplift of different crustal blocks (numbered as on Fig. 2) about 95 Myr ago. Ornamentation as on Fig. 2.

steep fractures and shear zones. Oscillatory vertical movements of these ribbon-like blocks controlled both the deposition of sediments and their near-surface deformation from at least Cretaceous to early Tertiary time.

The West Peruvian Trough was itself divided into western and eastern belts by another steep shear zone, the Tapacocha Axis¹⁴, which between 115 and 95 Myr ago allowed the western belt to subside about 4,000 m more than the eastern belt (Figs 1 and 2). The crustal blocks which underlie the western and

eastern belts of the West Peruvian Trough are here called the Paramonga and Chavin blocks, respectively, and those which underlie the Paracas and Marañón Geanticlines are called the Paracas and Marañón blocks, respectively (Fig. 2).

Casma Volcanics and the Coastal Batholith

The Casma Group of volcanic rocks was erupted through the descending Paramonga Block from the rising Coastal Batholith below, forming a pile of andesite lavas, sills and pyroclastic rocks 3,000–6,000 m thick (Fig. 2). Meanwhile, about 3,000 m of sandstone and limestone¹⁰ accumulated on the more slowly subsiding Chavin Block. Relative variations between the amount of volcanic material erupted and the rate of subsidence of the Paramonga Block resulted in oscillatory cycles of deeper and shallower marine and terrestrial volcanism within the Casma Group. The age ranges of ammonites interbedded with the volcanic sequence indicate that most eruptions occurred in the late Middle Albian and early Upper Albian (about 100 Myr ago)^{7,14}. The occurrence of granodiorite, diorite and gabbro fragments in some pyroclastic flows suggests that the Casma Group of volcanic rocks was erupted through, and was probably associated with, plutonic rocks similar to the Coastal Batholith which was later emplaced into the Casma Group.

The oldest intrusions of the Coastal Batholith are numerous bodies of hornblende gabbro and diorite, collectively called the Patap Complex⁷, which were emplaced into the eastern side of the Paramonga Block (Fig. 2). They contain more hornblende than pyroxene and seem to have crystallised from hydrous magmas. Many bodies of hornblende gabbro show rhythmic layering which may have formed in association with volcanoes within the upper part of the Casma Group.

Regional deformation

Intermittent pulses of deformation during intrusion of the Patap Complex folded the Casma Group into folds with vertical axial surfaces and sub-horizontal fold axes parallel with the Andes. The anticlines are mostly broad and open whereas the synclines are narrow and tight (Fig. 3). The gentle north-westerly plunge of the Canoa Syncline reveals that at structurally deeper levels to the south-east, the fold becomes even tighter and passes into a 2–4 km wide belt of isoclinal folds. The grade of metamorphism also increases progressively towards structurally deeper levels, from being hardly noticeable in the north-west where the structure is an open fold, to greenschist and amphibolite facies where the folds are isoclinal. The Canoa Syncline lies near, but is slightly oblique to, the western edge of the Coastal Batholith (Fig. 4), and a similar belt of strong deformation and syntectonic metamorphism occurs at the eastern edge of the batholith along the Tapacocha Axis (Fig. 3). These belts of deformation may be the upward expression of major shear zones in the crust below, which partly controlled the edges of the rising batholith, and were channels of high heat flow from the rising plutons.

The style of deformation of the contemporary rocks on the Chavin Block was completely different (Fig. 3). There, the sequence of limestone and sandstone was folded as a single unit by décollement on the underlying Lower Cretaceous Oyon Shale in the south, and the Jurassic Chicama Shale in the north^{9,10}. Upright ribbon-like folds with axes traceable for 100 km or more occur on the centre of the Chavin Block, whereas towards the margins of the block, the folds are overturned outwards, and lower fold limbs are increasingly attenuated and pass into outwardly directed thrusts.

To the east, the thin, Cretaceous sedimentary rocks and pre-Mesozoic basement of the Marañón Block were broken up by normal and reverse faults^{9,10} (Fig. 3).

The distinctions between the Paramonga, Chavin and Marañón blocks which had previously been marked by different kinds and thicknesses of sediments during differential rates of block subsidence, were thus emphasised by three distinct deformation styles during uplift of the blocks. The Paramonga Block was underlain by rising magmas of the Coastal Batholith

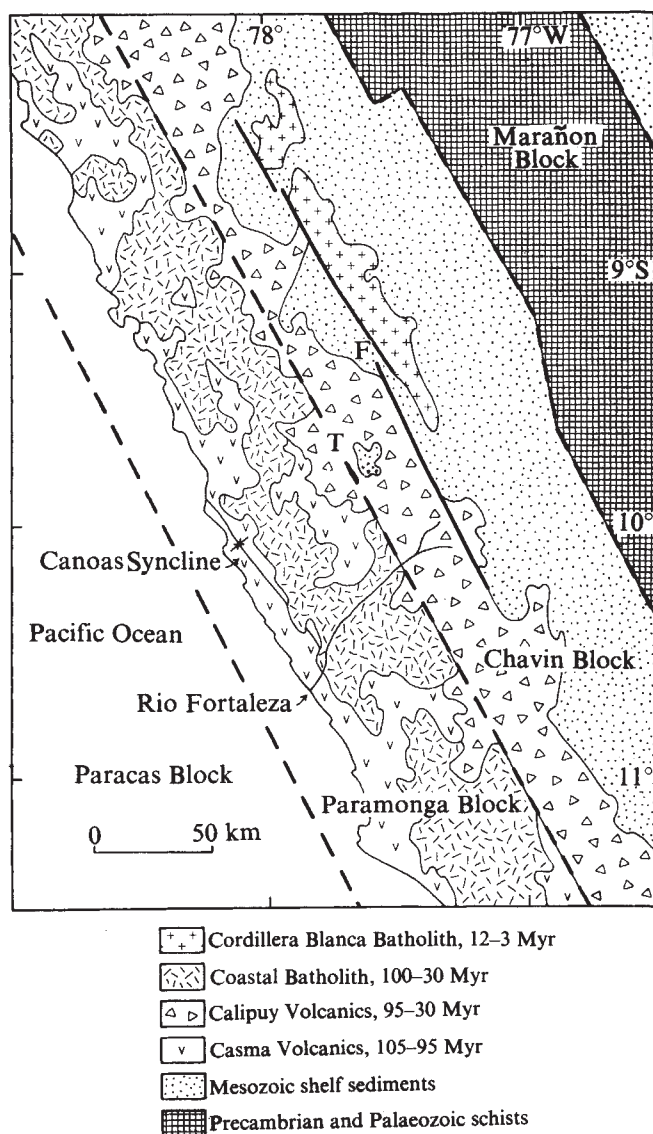


Fig. 4 Map of northern Peru showing the spatial association of the Coastal Batholith and Casma Volcanics, both restricted to the Paramonga Block, and the Calipuy Volcanics which spread on to the edge of the Chavin Block. Map simplified after ref. 15. T, Tapacocha Axis; F, fault.

and the folds represent the high penetration of ductile deformation above steep, basement shear zones associated with the rise of the batholith through the block. The Chavin Block to the east was not infiltrated by rising magma, and it rose intact and was tilted or arched, causing folding of the Cretaceous sediments which lay on it by gravity sliding on a lubricant of shale. Further to the east on the Marañón Block, the shale lubricant

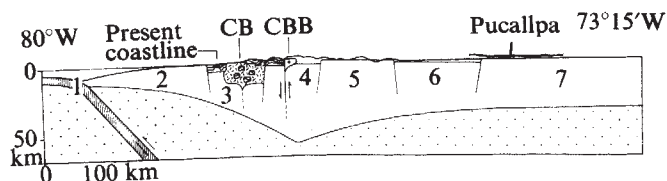


Fig. 5 Schematic section (A–B, Fig. 1) across Peru, showing the sum of Tertiary and Quaternary block movements, the location of the whole Coastal Batholith (CB) and Cordillera Blanca Batholith (CBB), and the present topography. Continental crust—white; mantle—stippled. Crustal blocks numbered as on Fig. 2; thickness of continental crust after ref. 16.

was absent and faults of the rigid basement penetrated the thin cover of sedimentary rocks. The overturned folds and outwardly directed thrusts at the eastern edge of the Chavin Block locally transgressed onto the Marañón Block and were dissected by the block faulting (Fig. 3). This suggests that the centre of the Chavin Block was raised higher than the Marañón Block, and that the folds flowed eastwards under the influence of gravity.

Calipuy Volcanics and the main intrusions of the Coastal Batholith

A 2,000–4,000 m thick terrestrial sequence of andesite, dacite and rhyolite, lava and pyroclastic flows, called the Calipuy Volcanics, lies unconformably on folded Casma Volcanics on the Paramonga Block, and spreads eastwards over the non-volcanic Cretaceous sediments on the western part of the Chavin Block (Fig. 4). It is intruded by many plutons of the Coastal Batholith and is probably the product of fissure and caldera eruptions from tonalite, granodiorite and granite magmas which formed much of the batholith.

The batholith is a tabular complex of plutonic rocks, 50 km wide and about 15 km thick which extends for over 1,100 km along the eastern part of the Paramonga Block (Fig. 4). It is made up of a large number of plutons of mainly tonalite, granodiorite and granite which were emplaced by repeated cauldron subsidence into the centre of the pile of Casma and Calipuy volcanic rocks to within 3 km or less of the surface¹². Individual plutons form ring dykes and rectangular bodies parallel with the continental margin, with steep walls and flat roofs and floors.

Chronology of volcanic and plutonic history

The Casma Volcanics were erupted 100–95 Myr ago. They immediately preceded, and locally overlapped in time with, the intrusion of the earliest, Patap Complex of the Coastal Batholith, and were probably derived entirely from magmas of the rising batholith¹². The Calipuy Volcanics which postdate uplift and erosion of the deformed Casma Volcanics and Patap Complex, were probably erupted from magmas which formed the younger complexes of the batholith 95–30 Myr ago (P. A. Wilson, unpublished). There was thus a close association between volcanic eruptions and plutonic intrusions in the eastern part of the Paramonga Block for at least 70 Myr during oscillatory vertical movements of the block.

Uplift and erosion of the modern Andes

The succession of erosion surfaces which are preserved on the western flank of the Andes indicate sporadic uplift of a gently undulating landscape formed by erosion of the Coastal Batholith and its volcanic envelope⁷. The erosion surfaces increase in altitude with increasing antiquity and distance from the western edge of the Paramonga Block. The steady increase in the slope of the erosion surfaces with age indicates that uplift occurred mainly by arching of the Chavin Block and westward

tilting of the Paramonga Block. Major uplift and erosion of the modern Andes occurred between the time of emplacement of the last major plutons of the Coastal Batholith 30 Myr ago (P. A. Wilson, unpublished) and the eruption of an ignimbrite down the aggraded ancestral Rio Fortaleza Valley 6 Myr ago (P. A. Wilson, unpublished). Later incision of the valley through this ignimbrite indicates further uplift of at least 500 m within the past 6 Myr.

In addition, the Chavin Block was split by a complex of steep major faults along the western foot of the Cordillera Blanca (Figs 4 and 5). The faults have been intermittently active from at least 6 Myr ago to the present time, and have raised the eastern part of the Chavin Block, which forms the crest of the Andes, a further 500–1,000 m. The fault complex may be the upward expression of a deep fracture–shear zone system which was active during late Tertiary time and which localised the ascending magmas that formed the Cordillera Blanca Batholith 12–3 Myr ago¹⁷.

Andean structure and plate tectonics

The Peruvian Andes do not show any evidence of the strong deformation of the continental margin which occurred⁴ in western North America during eastward movement of oceanic crust beneath the continent. In Peru the relative rigidity of the continent enabled it to float intact on the weaker oceanic crust which was deflected beneath it, and only weak tensile and compressive stresses were intermittently transmitted through the continental margin, causing oscillations of the crustal blocks. The steep fault and shear zones which bound the main crustal blocks are parallel with similar structures which were active during late Palaeozoic and early Mesozoic time, and they may all have been initiated in the same major tensile stress regime which during Precambrian time split the South American continent from continental crust to the west.

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letters to nature

Is Cir X-1 a runaway binary?

RUNAWAY stars are produced when there is a supernova explosion in a close binary system. Usually such an explosion disrupts the binary system, but Gott¹ has suggested that in a significant fraction of such supernovae the collapsed residue of the explosion may be retained in orbit around the runaway

second star, the resultant system being a binary with high eccentricity and large space velocity. We suggest here that Cir X-1 is such a system.

Figure 1 shows a 408-MHz radio map of the Cir X-1 region. The map was produced from observations made with the Molonglo Cross radio telescope, and is centred on the recently

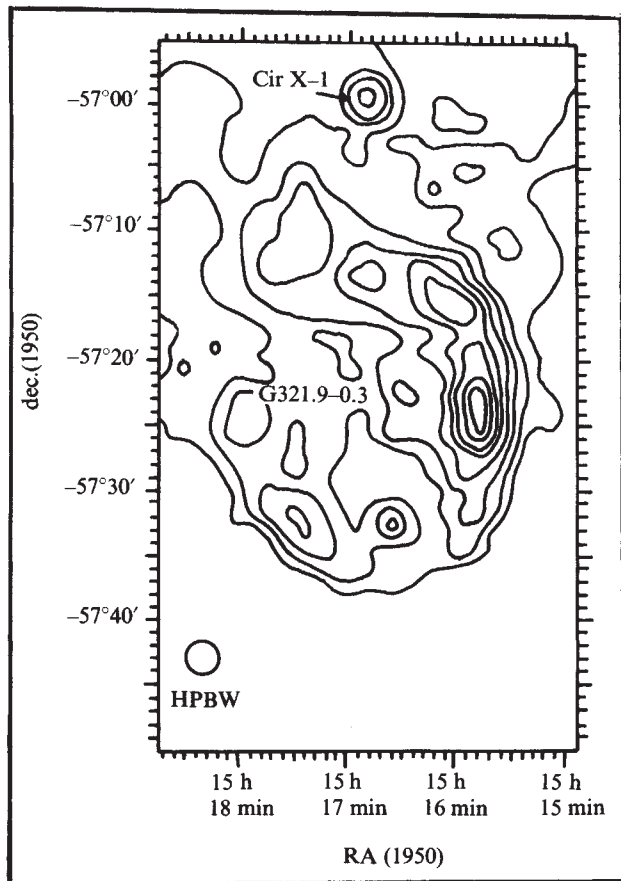


Fig. 1 Map of the supernova remnant G321.9-0.3 made at 408 MHz with the Molonglo radio telescope (half power beam-width, HPBW, as shown). The contour unit of brightness temperature averaged over the beam solid angle is 23.8 K (corresponding to 0.1 Jy for a point source).

discovered supernova remnant (SNR) G321.9-0.3 (refs 2, 3). Figure 1 also shows a point radio source approximately 7' north of the SNR boundary. The position and flux density estimates for the point radio source are given in Table 1.

The position of Cir X-1 has recently been revised by Jones *et al.*⁴ and the point radio source lies well within their 2' × 1' error box, approximately 16'' from their best position. We therefore suggest an association of the X-ray source Cir X-1 and the point radio source. Location of the radio source within the error box is not sufficient grounds for a definite association. The probability of finding a radio source of 0.5 Jy inside the X-ray error box is, however, approximately 1 in 3,000 (see ref. 5). The detection of correlated short term variability in the X-ray and radio sources, as found in several other similar systems, would secure the identification, and we plan to make such observations in the near future.

The radio flux density estimates give a spectral index (flux proportional to ν^α) $\alpha_{480}^{5,000} = -0.25$. This is an unusual spectrum for a point radio source (singling out the source from most extragalactic sources or galactic H II regions⁶). The radio source associated with Cyg X-1 also has an unusual spectral index, with a non-thermal spectrum below about 2,700 MHz and a spectral upturn at higher frequencies⁷. Between our measuring frequencies of 408 MHz and 5,000 MHz, a similar change of spectral index for the Cir X-1 radio source could explain the apparently shallow spectrum obtained. Cir X-1 and Cyg X-1 do differ, however, in one important respect: the ratio of radio to X-ray emission for Cir X-1 is at least an order of magnitude greater than that for Cyg X-1. An alternative explanation for the unusual radio spectral index obtained for Cir X-1 could be long

term variability of the radio source, since the 5,000 MHz observations were made 18 months later than those at 408 MHz; but such variability would in itself make the source of considerable interest. A possible pulsar-like behaviour of the radio source has been investigated at Molonglo, without success (J. Sutton, personal communication). A pulsar with period less than 160 ms, or a dispersion measure greater than 500, would not have been detected in this search.

Are Cir X-1 and the SNR G321.9-0.3 related and, in particular, is Cir X-1 a runaway system ejected from the supernova? A distance estimate for the SNR may be inferred from comparison of its surface brightness and angular diameter with those SNRs with well determined distances. Using such a technique, Clark and Caswell⁸ derive the following parameters for G321.9-0.3: linear diameter 39 pc, distance from Sun 5.5 kpc, distance from galactic plane -29 pc. In spite of limitations in the technique, the uncertainty in these parameter estimates is believed to be less than $\pm 30\%$. The estimate of age for SNRs, however, remains particularly uncertain; a comparison of the surface brightness of G321.9-0.3 with the values for those few remnants of known age suggests a possible age range of 2×10^4 to 10^5 yr. These parameters then give a required average transverse velocity for Cir X-1, if it were indeed created in the supernova, of 10^2 - 10^3 km s⁻¹, assuming the centroid of the radio remnant gives the position of the initial supernova explosion.

The properties of Cir X-1 are in fact compatible with it being a runaway binary at a distance of ~ 5 kpc and with an age of $\sim 10^4$ - 10^5 yr, as shown below.

Until an optical counterpart is discovered, the distance to Cir X-1 must remain uncertain, and difficult to estimate. X-ray spectra of Cir X-1 are strongly attenuated^{9,10} at energies ≤ 2 keV. Below approximately 6 keV the spectra can be fitted with an exponential (thermal Bremsstrahlung) spectrum with a temperature in the range 3.5×10^7 - 4.3×10^7 K and a hydrogen column density 2.5×10^{22} - 2.9×10^{22} cm⁻². It is likely that a considerable fraction of the attenuation is circumstellar; however, if we assume it is all interstellar we obtain an upper limit to the distance of 9 kpc taking an average interstellar density of 1 H atom cm⁻³.

Jones *et al.*⁴ have reported that there is no star brighter than 15 mag in their error box. By considering stars of various spectral types we can set a lower limit on the distance to the source by taking the average visual absorption to be 1.5 mag kpc⁻¹ for this direction in the galactic plane. Five of the eight variable X-ray sources with known optical counterparts have been identified with binary systems having early type stars as primaries. For a B0 supergiant, consistent with its not being brighter than 15 mag, a lower limit to the distance is 5.0 kpc; for an A0 supergiant the distance drops to 4.5 kpc and for a B0 main sequence to 4.0 kpc. For later stars the minimum distance decreases accordingly.

The distribution of 2-10 keV luminosities for galactic X-ray sources has been investigated by Margon and Ostriker¹¹ who found a range of values between 10^{36} and 10^{38} ergs s⁻¹ with a peak near 5×10^{37} ergs s⁻¹. Taking the 2-10 keV Uhuru maximum count rate of 720 s⁻¹ (1 count s⁻¹ = 1.7×10^{11} erg cm⁻² s⁻¹) gives a distance range of 1 to 9 kpc with the most likely distance, corresponding to the peak of the distribution, at 6 kpc. We conclude that the data now available for Cir X-1 are not inconsistent with it being at the distance of the SNR (5.5 kpc). Further improvements in defining this distance are clearly needed, but must await an optical identification.

By analogy with all of the other galactic variable X-ray sources Cir X-1 is likely to be a binary system, the X-ray production being due to accretion onto a collapsed object. Although observations of X-ray 'eclipses' have been reported^{4,12}, no unique binary period has yet been found, Jones *et al.*⁴ claiming that if a binary period exists it must be greater than 12 d.

If Cir X-1 originated in the supernova with remnant G321.9-0.3, it is likely that the explosion increased the eccentricity and the period of the binary system. For a system with a large eccentricity and long period, the accretion process may break

Table 1 Point source data

	RA (1950)	dec. (1950)	Flux density (Jy)
408 MHz (Molonglo)	15 h 16 min 47.3s	— 56° 59'44"	0.46
5,000 MHz (Parkes)	15 h 16 min 46.4s	— 56° 59'54"	0.25

The 408 MHz and 5,000 MHz positional estimates have 12" and 20" standard errors respectively.

down for a time around apastron giving what seems to be an eclipse in the light curve and so hampering the determination of a binary period. Such a determination would also be hampered if the system is precessing so that it does not seem to eclipse on each orbit. It is likely that after some considerable time tidal forces will tend to circularise the orbit¹³, as observed in such a system as Cen X-3 which is believed to be 10⁵–10⁶ yr old. Without an identified period, it seems possible that Cir X-1 is a relatively young system, 10⁴–10⁵ yr old (the estimated age of the SNR), whose orbit has not been circularised as in the older, better understood binary systems. This is the first possible SNR–binary system association to be discovered and gives credibility to theories suggesting that supernovae have given rise to all the close binary systems, if not all the galactic X-ray sources.

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Note added in proof: An optical plate taken by A. J. Longmore and R. D. Cannon with the UK 48-inch Schmidt telescope at Siding Spring, Australia, shows that there are two stars brighter than 20 mag within 20" and five stars within 30" of the best 408-MHz position.

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passing through the centre of the neutron star at an angle to the rotation axis. Such a field is modelled nicely by a magnetic dipole of moment μ located at the centre and oblique to the axis of rotation. An oblique dipole rotator generates electromagnetic radiation at the rotation frequency and the energy radiated is at the expense of the rotational kinetic energy¹. The rotation period consequently increases, in rough agreement with observation.

Various sources of evidence suggest that the oblique dipole rotator is an oversimplified model. Recent studies of magnetic variable stars^{2,3} have shown that an inclined magnetic dipole moment, displaced from the rotation axis, provides an improved model for reproducing the surface field. Displacements of 0.1 or more of a stellar radius are not unusual. Of the six pulsars^{4,5} known to have interpulses well separated from their main pulses, three (PSR0823+26, PSR0904+77, PSR1929+10) have weak interpulses located midway between their main pulses. The magnetic dipole moment for these pulsars is perpendicular to the spin axis, and is possibly displaced from the centre in directions parallel and perpendicular to the spin axis. If the pulsed luminosities are proportional to the square of the field intensity, then the displacements from the spin axis are approximately one-third of the radius of the neutron star. The other three (PSR0531+21, PSR0950+08, PSR1055-51) have strong interpulses located asymmetrically at 140°–150° from their main pulses. The field configurations of these pulsars are also easily reproduced by a dipole moment, in a plane perpendicular to the spin axis, and offset from the spin axis a distance that is again about one-third the radius of the neutron star. It is of interest to note that Earth and Jupiter also have oblique off-centred dipole fields. All known observations suggest that planetary and stellar objects are generally oblique off-centred dipole rotators.

We consider, then, a pulsar with a magnetic dipole moment that is arbitrarily oriented and also displaced from the rotation axis. In cylindrical coordinates the components of the dipole moment are μ_z , μ_ρ , μ_ϕ , where the z coordinate is along the rotation axis. Unlike an oblique centred dipole rotator that radiates only at the rotation frequency Ω , the oblique off-centred dipole rotator radiates at all harmonics of Ω . Moreover, the radiation field is not that of a pure dipole, but consists of a superposition of both magnetic and electric multipoles of all orders. If s is the distance of the dipole from the rotation axis, then when $s\Omega/c \ll 1$, most of the energy is emitted at the fundamental frequency and the total low frequency luminosity is approximately

$$L = \frac{2\Omega^4}{3c^3} \left[\left(\mu_\rho^2 + \mu_\phi^2 \right) + \frac{2}{5} \left(\frac{s\Omega}{c} \mu_z \right)^2 \right] \quad (1)$$

The first term on the right, enclosed in the parentheses is the power radiated by a simple dipole rotator, whereas the second term in parentheses represents the power radiated by the sum of the electric-dipole and magnetic-quadrupole fields.

In addition to an increase in luminosity, we find that the luminosity averaged over a rotation period is asymmetric about the rotational equatorial plane. This asymmetric emission is the result of interference of magnetic-dipole radiation fields with electric-dipole and magnetic-quadrupole radiation fields. The asymmetric emission of low frequency electromagnetic radiation results in a net reaction force on the neutron star that is approximately

$$F = -2s\Omega^5\mu_z\mu_\phi/15c^5 \quad (2)$$

in the direction of Ω . On writing $F = \epsilon L/c$, we have

$$\epsilon = - \frac{s\Omega\mu_z\mu_\phi/c}{5(\mu_\rho^2 + \mu_\phi^2) + 2(s\Omega\mu_z/c)^2} \quad (3)$$

for the 'radiation asymmetry coefficient'. We note that ϵ has a maximum value of 0.16 when $\mu_\rho = 0$, $\mu_\phi = 0.63s\Omega\mu_z/c$. Also,

Acceleration of pulsars to high velocities by asymmetric radiation

HERE we propose that pulsars are accelerated by the emission of asymmetric low frequency electromagnetic radiation and rapidly attain characteristic velocities exceeding 100 km s⁻¹.

Pulsars are believed to be rotating neutron stars that have intense surface magnetic fields. The structure of the field is often assumed to be dipolar, with the magnetic axis of symmetry

when a pulsar is young, $s\Omega/c$ may be a significant fraction of unity and therefore values of $\varepsilon \sim 0.1$ are quite possible.

For a young and rapidly spinning pulsar the luminosity and radiation force are both large, and it is possible that the radiation force rapidly accelerates the whole pulsar to a high velocity. The acceleration of the pulsar is

$$dV/dt = \varepsilon L/Mc \quad (4)$$

and since εL is proportional to Ω^5 , maximum acceleration occurs on the time scale $I\Omega_0^2/2L_0$, where I is the angular moment of inertia and the zero subscript denotes initial values. Thus, the velocity of the pulsar, as a result of asymmetric emission of radiation, is

$$\Delta V \sim \varepsilon_0 I \Omega_0^2 / 2Mc \quad (5)$$

For a neutron star of radius 10 km this gives $\Delta V \sim 10^{-4} \varepsilon_0 \Omega_0^2 \text{ km s}^{-1}$, and for initial values $\varepsilon_0 \sim 0.1$, $\Omega_0 \sim 10^4$, the final velocity is $\sim 10^3 \text{ km s}^{-1}$. It is evident that with reasonable values of ε_0 and Ω_0 this radiation acceleration mechanism is efficient and capable of explaining the remarkably high velocities of many pulsars. For example⁶, PSR113+16 has large proper motion that is equivalent to a velocity in excess of 380 km s^{-1} , if the pulsar is at a distance of 120 pc. (In our analyses (to be published) we find evidence for pulsar velocities exceeding 700 km s^{-1} .)

Most pulsars are probably born in binary systems, and it can be shown that the mass ejected from close binary systems in a supernova event is generally inadequate to unbind the binary members⁷. It can be shown, however, that the radiation reaction force is initially sufficiently strong to wrench apart all but the closest binaries, thus explaining why most pulsars are not members of binary systems. In the case of those pulsars that survive as members of binary systems (for example, the Hulse-Taylor pulsar⁸, PSR1913+16), the radiation force plays a dominant role in the evolution of the orbital parameters.

The theory of radiative acceleration of pulsars with its various ramifications and implications will be presented elsewhere.

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Comets and interstellar masers

RECENT advances in our understanding of the origin of the Solar System have led to renewed speculation on the origin of comets^{1,2}. Oort³ proposed that long-period comets originate in a cometary cloud about 10^5 AU from the Sun and Cameron⁴ has suggested that this cloud has its origin in association with the primordial solar nebula. Other investigators have placed the origin in the interstellar medium⁵.

Whipple and Lecar⁶ have suggested that long-period comets arise from the interaction of the primordial solar nebula with the interstellar medium. The opposing action of the solar wind from the new star and the interstellar radiation field squeezes the excess nebular material into a ring at the nebular edge

where saturated molecules such as H_2O and heavy metal compounds condense.

High density rings around young stellar and protostellar objects at heliocentric distance $r = 10^4$ – 10^5 AU have also been suggested as the location of interstellar masers⁷. Because these objects contain large fractional abundances of H_2O and perhaps other saturated molecules such as CH_3OH , and exist in an environment believed to be favourable to comet formation, there may exist an ontological relationship between masers and comets. I suggest that the long-period comets are among the condensed remnants of cooled interstellar masers formed in a dense region at the nebular boundary. I shall discuss the dynamics of the maser-comet transition and suggest ways in which this relationship may be exploited to expand our understanding of star and planet formation.

Maser radiation from OH, H_2O , SiO, and perhaps CH_3OH has been observed from small knots of gas and dust in the direction of molecular radio sources associated with H II regions, and also towards certain infrared stars⁷⁻⁹. The most common source of maser radiation, the OH radical, is often associated with H_2O maser radiation and the H_2O maser is always closely associated with OH-maser sources⁷. The sizes of the emitting regions vary from 10 AU for H_2O regions to 10^3 AU or greater for OH regions. Theoretical considerations of the pumping mechanism place limits on the density of H_2 in these regions of $10^6 \leq n(\text{H}_2) \leq 10^9 \text{ cm}^{-3}$ (refs 7 and 10). Fractional abundances for OH of 10^{-6} and for H_2O of 10^{-5} – 10^{-4} are suggested. The H_2O region may be contiguous with the OH region but higher in density and temperature, and perhaps closer to the central star.

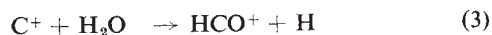
It has been demonstrated (J. C. Weisheit, A. Dalgarno and M.O., unpublished) that the chemical process



is the only source capable of providing the large abundance of OH. H_2O is formed by



and by ion-molecule reactions initiated by penetrating cosmic rays. H_2O is removed slowly by the reverse reaction (2) and more rapidly by^{11,12}



and



Other saturated molecules are removed by analogous processes. Photons which ionise carbon and dissociate H_2O do not penetrate much beyond $10^{21}/n \text{ cm}$ into a region with the normal interstellar dust-to-gas ratio. The residual ionisation of C^+ from cosmic rays is small and, except at the edges of the maser region, most of the oxygen is bound into H_2O and other molecules for $T > 200 \text{ K}$. The lifetime of H_2O is greater than 10^{12} s , except at the edges of the region, even if it lies near an O-type star. Unsaturated chemical species are removed much more rapidly by reactions among themselves such as



with time scales of $10^{14}/n \text{ s}$ (ref. 11).

The elevated temperatures needed to produce masers are obtained in or near protostellar objects and young stars. Observational evidence and theoretical considerations suggest that some masers exist in rings of hot dense gas on the edge of protostellar objects and nebulae associated with young stars⁷. A shock front related to the infall of material into a new star or the interaction of the solar wind of a new T-Tauri star with the remnant nebular gas may generate maser action at several

points circumferential to the object. Many maser point sources exhibiting variations on time scales of the order of a year are found associated with single compact H II regions which may be cocoon stars.

Consider the thermal evolution of maser sources that lie on the edge of the nebula of a new star. The chemical scheme and the theoretical excitation mechanism suggest $T \geq 300$ K in an OH-H₂O maser region⁷. There is probably an excess of dust by comparison with the interstellar value because of accumulation at the nebular edge. Carbon monoxide, initially present in substantial amounts in the interstellar medium (M.O. and A. Dalgarno, unpublished), may be converted to CH₄ and other saturated molecules at the elevated temperatures of the maser. Eventually, the shock wave that heats the region dissipates or moves on, and the maser cools. Maser fluctuation periods and shock speeds indicate a lifetime for the hot phase of 10^8 – 10^{10} s. Cooling proceeds through emission from grains and in molecular transitions. A lower limit on the cooling rate is found from the cooling through thermalised CO transitions. For the velocity gradients, temperatures, and densities typical of these regions, the cooling time is $10^3 n$ s. (T. de Jong, S.-I. Chu and A. Dalgarno, unpublished). H₂O will itself cool the gas very efficiently. Conversion of CO to hydrogenated species increases the cooling rate¹³. Conceivably it is this conversion which shuts off the maser.

The time scale for cooling is much shorter than that for chemical removal of the saturated species. H₂O, NH₃, CH₄ and other saturated molecules are frozen in with their maser-region densities for $n \lesssim 10^9$ cm⁻³ at depths where ultraviolet photons do not penetrate. On the other hand, OH, O, N and other chemical radicals disappear during cooling.

The presence of H₂O and any other molecular hydrides increases the cooling rate in the maser region significantly as compared with the surrounding medium, even after maser action ceases. The resulting pressure gradient and decrease in volume of the H₂O region may lead to a greatly enhanced density. Simultaneously, the saturated species will accrete on to grains as they cool with the gas. The time scale for accretion is $10^{17}/n$ s in the interstellar medium¹⁴, and probably shorter in the outer regions of the nebula. Thus the accretion of ice mantles of saturated species proceeds before they are chemically removed. The combination of large pressure gradients and competing solar-wind and interstellar-photon pressure⁶ may bring about the enhanced density of grains needed for accumulation of the matter into a comet⁴. The mass of condensable elements in a cylindrical maser⁷ of radius r_m and depth l is

$$10^{-3} \alpha \delta n M (\pi r_m^2 l) \quad (7)$$

where M is the average molecular weight, δ the depletion factor and α the degree of conversion to condensable molecules for minor constituent elements. In an H₂O-maser region⁷ with $r_m = 5 \times 10^{13}$ cm, $l = 3 \times 10^{15}$ cm, $n = 10^6$ cm⁻³, $M = 20$ and $\delta \sim \alpha = 1/3$, the condensed mass is about 6×10^{22} g; this corresponds to a comet nucleus of radius 200 km with density of 1 g cm⁻³, reasonably close to the estimated values for bright comets within the uncertainties of the model. For lower values of α and δ , or larger density (representing a different chemical evolution), a smaller comet is generated. I have chosen a maser of relatively low density. Masers of higher density may fragment into several comets. The total mass of condensables available in a ring of density 10^6 cm⁻³ and width 10^{16} cm at a distance of 3×10^{17} cm from the primordial Sun corresponds to 10^9 large comets, enough to explain the observed frequency of appearance of new comets². This density is in accord with values derived for the maser region in the Orion nebula¹⁰ at about 10^{17} cm from the HCN emission peak.

If the postulated maser-comet relationship is correct, information on the origin and composition of comets can be obtained from observations of interstellar masers, and of masers from observations of comets. The chemical imprint in comets of the early stages of the formation of the Solar System¹⁸

may yield information on the temperatures and densities at that epoch for the outer region of the nebula. As an example, I shall consider the equilibrium involving CO, CH₄, H₂ and H₂O in comet and maser regions. Donn¹⁵ has suggested that this equilibrium may serve as an indicator of the temperature in the gas when the comet formed. The overall reaction is



The forward direction is favoured over the reverse for $T < 1,000$ K (refs 16 and 17), but the rate-determining steps are much slower than the probable thermal time scales in a maser region. None of the gas-phase reaction schemes proposed for the interstellar medium produce CH₄ in abundances comparable to CO^{11,18}. Observational and theoretical evidence (M.O. and A. Dalgarno, unpublished) suggests that 10–100% of the carbon atoms are found in CO in dense interstellar clouds. Therefore, unless CH₄ is produced efficiently on grain surfaces¹⁴, one does not expect

$$n(\text{CO})/n(\text{CH}_4) \ll 1$$

in interstellar clouds. Since CH₄ is not produced in the cometary coma¹⁹ that ratio should not be much different in comets unless the parent maser region has experienced a high temperature phase of evolution.

Similarly, H₂O is clearly in substantial abundance in the maser region. Since H₂O is not removed during cooling, the H₂O should contain a substantial fraction of the oxygen in the cometary coma. Contrary evidence would suggest heating of the parent region to $T > 2,000$ K (ref. 17), or penetration of that region by dissociating photons. Interpretation of chemical inprints is complicated by the fact that comets originating on the outer edge of the maser ring, where photons penetrate, will have a different thermal and ionisation history from those formed deep within the ring. In addition, observed masers may occur near stars with mass greater than $1 M_\odot$, whereas our comet observations are restricted to the solar neighbourhood. In spite of these complications, the maser-comet relationship may be a useful tool in understanding the early Solar System.

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Invalid 4.01-Gyr model U-Pb 'age' of the Nakhla meteorite

NAKHLA, an achondrite composed dominantly of the minerals 'diopside' and olivine¹, is possibly a unique meteorite. It has a ³⁹Ar–⁴⁰Ar age of about 1.3 Gyr (refs 2 and 3) and recently its Rb–Sr age was shown by Papanastassiou and Wasserburg⁴ to be similar. In their discussion, these authors⁴ use the concept

Table 1 U and Pb concentrations and Pb isotopic composition in samples of Nakhla

Sample	Weight (g)	U (p.p.m.)	Pb (p.p.m.)	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	Blank* (%)	μ †
Chip A	0.88883	0.0528	0.5532	13.780 ± 0.020	12.331 ± 0.020	34.019 ± 0.050	0.5	5.04
Chip B	0.77983	0.0486	0.5238	13.566 ± 0.010	12.187 ± 0.010	34.712 ± 0.030	0.6	4.93
Whole meteorite powder	0.50644	0.0372	0.3521	13.588 ± 0.030	12.204 ± 0.030	34.120 ± 0.080	1.4	5.56

The data are corrected for analytical blanks, and the errors quoted (at the 1 σ level) include uncertainties from the blank correction.

*Pb blank divided by the total Pb in the sample used.

†Measured $^{238}\text{U}/^{204}\text{Pb}$ ratios.

of model Rb–Sr ‘ages’ as part of their argument that Nakhla originated in a differentiated planetary body not unlike the Earth, and in particular to argue that this parent body suffered gross differentiation between 3.6 and 1.37 Gyr ago. We show that the U–Pb systematics of Nakhla are inconsistent with one or more of the assumptions required for the calculation of a model U–Pb ‘age’, thus indicating that model ‘ages’ for other radioactive decay schemes may be generally invalid.

Table 1 shows U and Pb contents and the atomic ratios $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ determined for each of three bulk samples of Nakhla. The techniques used are described elsewhere^{5,6}; U concentrations are determined to about $\pm 1\%$ and Pb concentrations to about $\pm 1.5\%$. Blank levels were about 0.05 ng U and 2 ng Pb. It is clear that single stage model ‘ages’ are close to 4.01 Gyr (Table 2); indeed the mean model ‘age’ is 4.01 ± 0.09 Gyr, where the error quoted is twice the standard

Table 2 Radiogenic $^{207}\text{Pb}/^{206}\text{Pb}$ ratios and single-stage model ages for Nakhla

Sample	Single-stage ‘ages’ in Gyr			
	$^{207}\text{Pb}/^{206}\text{Pb}_R$	$^{207}\text{Pb}/^{206}\text{Pb}$	$^{206}\text{Pb}/^{238}\text{U}$	$^{207}\text{Pb}/^{235}\text{U}$
Chip A	0.45540	4.103	4.096	4.100
Chip B	0.44447	4.067	4.014	4.049
Whole meteorite powder	0.44616	4.073	3.681	3.938

error of the mean. In these calculations the initial Pb isotope ratios are Tatsumoto’s revised values for Pb in the troilite (FeS) of the Cañon Diablo iron meteorite⁷, and the latest values for the U decay constants are used⁸.

Model ‘age’ calculations are used when the initial isotopic ratio of a daughter element cannot be obtained directly from measurements; for example, in the U–Pb and Rb–Sr systems ‘primitive’ Pb and Sr isotopic ratios of meteorites have been combined with lunar, terrestrial and meteoritic measured values to obtain the ‘age’ of the early lunar differentiation^{9–11}, the ‘age’ of the Earth^{12,13} (U–Pb only) or the ‘ages’ of certain stony meteorites⁷, respectively. The various assumptions which must hold for a model ‘age’ calculation to be valid when the U–Pb system is used, are as follows.

(1) The Pb isotope ratios of Cañon Diablo troilite represent the initial ratios of the material being studied.

(2) The ratio $^{235}\text{U}/^{238}\text{U}$ has changed with time by radioactive decay only, and is at present 1:137.88.

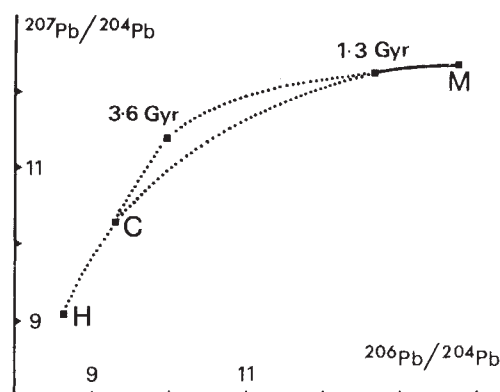
(3) Changes in Pb isotope ratios were brought about by U decay only.

(4) The system has remained closed, that is, the U/Pb ratio has not changed by chemical fractionation.

Many meteorites suffered a thermal event about 4.56 Gyr ago^{14,15} and Pb isotopic dating is broadly consistent with the assumption that Cañon Diablo troilite has retained the Pb isotopic ratios which generally obtained at that time¹⁶. If Nakhla is composed of matter which evolved in the Solar

System, as did the stuff of other meteorites, then 4.56 Gyr ago, it too, or its parent body, should have had the same Pb isotopic composition as other meteorites. Lead of the isotopic composition found in Cañon Diablo troilite could, however, not have evolved in a single stage into modern Nakhla Pb in a time other than about 4.01 Gyr unless the isotopic composition of its U was different from the accepted one (see (2)) and/or it had preferentially lost or gained ^{207}Pb or ^{206}Pb , that is, either or both of assumptions (2) and (3) did not obtain. If Nakhla experienced a two-stage history, it could have evolved from the Cañon Diablo composition at 4.56 Gyr, but only provided that the first stage had a U/Pb ratio of zero (usually expressed as zero μ , $^{238}\text{U}/^{204}\text{Pb}$) and the second stage lasted for about 4.01 Gyr. This implies that the Nakhla parent body was free of U from 4.56 to 4.01 Gyr, when U was introduced without the introduction of radiogenic Pb. This seems improbable. It is possible to invent three-stage (or higher order) histories which allow Nakhla to evolve to its present U and Pb content and Pb isotopic composition from the Cañon Diablo composition over 4.56 Gyr, but we have no evidence of such histories. In any case assumption (4) would have been violated. Thus, if assumptions (2), (3) and (4) did hold, the initial isotope ratios of Nakhla or its parent body were different from Cañon Diablo troilite Pb (assuming that this composition is linked to the time 4.56 Gyr); that is, assumption (1) did not hold. For Nakhla, at least one of assumptions (1)–(4) is false.

Fig 1 Nakhla (chip A) Pb growth: two interpretations. *a*, Single stage, curve HCM; observed $\mu = 5.04$. Growth begins 4.56 Gyr ago from H with hypothetical primitive values, $^{207}\text{Pb}/^{204}\text{Pb} = 9.1$ and $^{206}\text{Pb}/^{204}\text{Pb} = 8.6$, passes through C, the Cañon Diablo point, 4.1 Gyr ago and continues to M, with present-day measured ratios. *b*, Three-stage curves (diagrammatic) C–3.6 Gyr–1.3 Gyr–M. (1) From Cañon Diablo values, growth begins 4.56 Gyr ago with $\mu = 1.81$ till 3.6 Gyr ago, when $^{207}\text{Pb}/^{204}\text{Pb} = 11.0$ and $^{206}\text{Pb}/^{204}\text{Pb} = 9.8$. (2) Growth continues with $\mu_2 = 5.46$, till 1.3 Gyr ago, when $^{207}\text{Pb}/^{204}\text{Pb} = 12.3$ and $^{206}\text{Pb}/^{204}\text{Pb} = 12.7$. Here the single-stage curve is rejoined. (3) From 1.3 Gyr ago, with measured $\mu_3 = 5.04$ till the present. We emphasise that we have no evidence for selecting any particular three-stage model, nor do we have evidence for the hypothetical primitive point.



The simplest, single-stage explanation is that the Nakhla Pb isotopic ratios grew by U decay over the past 4.56 Gyr from Pb more primitive than that of Cañon Diablo troilite and with the following approximate ratios: $^{207}\text{Pb}/^{204}\text{Pb} = 9.1$ and $^{206}\text{Pb}/^{204}\text{Pb} = 8.6$ (Fig. 1). The existing U–Pb data are insufficient to prove whether or not this explanation is valid for Nakhla, or to reconcile it with the evidence from the Rb–Sr system⁴; it is mentioned here merely as a possibility. By chance, Papanastassiou and Wasserburg's interpretation of their Rb–Sr data⁴ could be compatible with a three-stage model based on our U–Pb data and Cañon Diablo initial lead isotopic ratios at 4.56 Gyr; U–Pb fractionation could have occurred at about 1.37 and 3.6 Gyr (Fig. 1). The second event, which these authors interpreted as representing internal redistribution of Rb and Sr, must, however, necessarily have changed the μ value in the bulk meteorite. It seems likely that any major change in the U/Pb ratio would also be accompanied by change in the Rb/Sr ratio so obliterating the record in the Rb–Sr system. The choice of 3.6 Gyr as the time for the earlier fractionation must therefore be arbitrary and is not specifically required by the U–Pb data. Indeed, we have established a 16 point internal isochron for the Nakhla Rb–Sr system which confirms this interpretation (to be published).

Because the Rb–Sr system has a single parent and single daughter isotope, in theory any value of the ratio $^{87}\text{Sr}/^{86}\text{Sr}$ could have developed by Rb decay from any less radiogenic initial ratio. Thus the applicability of a particular 'initial' $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of meteorites, such as BABI¹⁷, to determining model 'ages' (which, in the literature, are almost always single-stage 'ages') of other materials can be neither proved nor disproved. It seems likely, however, that Rb–Sr model 'ages' might well suffer from uncertainties similar to those shown to exist in the U–Pb systematics of Nakhla, and so it is desirable that meteorite chronologies be based on internal isochrons using both the Rb–Sr and U–Pb systems.

The systematics of Nakhla clearly demonstrate that the widely accepted 'primitive' Pb isotope ratios of Cañon Diablo troilite cannot represent initial values for single-stage evolution of the U–Pb systems of this other meteorite. The use of Cañon Diablo Pb in determining the 'ages' of lunar rocks (see, for example, ref. 11), the 'age' of the Earth^{13,18,19} or the 'ages' of stony meteorites^{19,20} by single-stage calculations might also be invalid. We are merely reiterating a reservation already expressed by numerous earlier workers (see, for example, refs 11, 13, 19 and 20), but we do so with added proof.

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Ages of fossil bones from British interglacial sites

THE time gap between the upper limit of radiocarbon dating ($\approx 60,000$ yr BP) and the lower limit of dates generally obtainable using the K–Ar method ($\approx 250,000$ yr BP) accounts for the scarcity of dates for the last two interglaciations (the Ipswichian and Hoxnian of Britain; the Eemian and Holsteinian of northern Europe). Accordingly, the ages of such important fossils as the Swanscombe and Steinheim skulls can only be guessed at. For that reason, the adaptation of a method that may date these interglacial periods is highly desirable. We discuss here the application of a uranium-series dating technique pertaining to that span of time.

Results were obtained for six samples of fossil bones from British interglacial sites (Table 1). Fossil bones can be dated using the uranium-series method by taking measurements of uranium isotopes and their long-lived daughters, ^{230}Th and ^{231}Pa (refs 1–4). The bone samples described here were cleaned by scraping and by ultrasonic scrubbing. The samples were crushed to a fine powder and ignited at 800°C for about 6 h. The abundances of uranium and thorium were determined using mass spectrometry; the $^{234}\text{U}/^{238}\text{U}$ and $^{230}\text{Th}/^{234}\text{U}$ activity ratios were determined using α spectrometry⁵. The $^{231}\text{Pa}/^{235}\text{U}$ activity ratios were determined using neutron activation and α spectrometry⁶.

Sample localities, analytical data, and calculated ages are given in Tables 1 and 2. The uranium content of the bones varies from 23 to 67 p.p.m. The concentration of common thorium, ^{232}Th , is low in all the samples; therefore, no correction for the initial ^{230}Th component is required. The measured $^{230}\text{Th}/^{234}\text{U}$ ratio yields a ^{230}Th date of $245,000 \pm 35,000 - 25,000$ yr BP for sample 37 from the Clacton locality. The $^{231}\text{Pa}/^{235}\text{U}$ ratio in this sample is 1.00 ± 0.04 , indicating that this fossil apparently remained a closed system with respect to uranium and its daughter elements, ^{230}Th and ^{231}Pa , during at least the last 150,000 yr of its history. The calculated ^{230}Th date therefore seems to be reliable.

Table 1 Sample descriptions

Sample 37, Clacton, Essex (OS ref. TM 157134): Find no. 243 of 1969 Clacton Golf Course Excavations (University of Chicago), cutting xii, layer 4. Sample from gravel below variegated marl, 1.47 m below surface^{12,22}. Gravel contains typical Clactonian assemblage and Clacton fauna; equals gravel under variegated marl in Oakley's 1934 excavation, but here shelly sand with *Valvata antiqua* separates the gravel and the marl.

Sample 38, Barnfield Pit, Swanscombe, Kent (OS ref.: TQ 598742); collected from lower gravel. Lower Barnfield stage of Oakley.

Sample 39, Barnfield Pit, Swanscombe. "A few cm below skull horizon". Base of upper middle gravel, containing rich fauna and Acheulian assemblage¹³. Middle Barnfield stage of Oakley.

Sample 63, Barnfield Pit, Swanscombe. Find no. 82 in 1972 excavations²³. Trench C 3, 1.88 m below datum. Middle of Lower Loam, 90 cm below top and about 110 cm above top of lower gravel. Lower Barnfield stage.

Sample 41, Stutton, Suffolk (OS ref.: TM 149338). From Brickearth 50–60 cm above beach and immediately above mollusca-bearing levels²⁴. Strata in ascending order: 0.3 m OD pollen of zone III, Ipswichian; 0.6–0.9 cm OD brackish mollusca; 1.0 m OD *Corbicula* maximum; 1.0–2.0 m OD *Corbicula* decline; level of bone sample about 2.5 m OD.

Sample 42, Brundon (Jordan's pit), Essex; close to Suffolk border (OS ref.: TL 860420). Sample marked "21.2.13 pit by railway towards Brundon". Fossil mammals occur in gravel with freshwater mollusca including *Corbicula*, and are underlain by till²⁵. Mollusca and mammals represent temperate conditions, but include much mammoth. Stone artefacts include hand axes and tortoise core.

Table 2 Analytical data and calculated dates of fossil bones from British interglacial sites

Sample	Locality	U (p.p.m.)	Th (p.p.m.)	$\frac{^{234}\text{Th}}{^{238}\text{U}}$	$\frac{^{230}\text{Th}}{^{234}\text{U}}$	^{230}Th date (yr)	$\frac{^{231}\text{Pa}}{^{235}\text{U}}$	^{231}Pa date† (yr)	Uranium series date (yr)
37	Clacton	45.5 ± 0.5	<0.03	1.26 ±0.02	0.946 ±0.038	245,000 +35,000 - 25,000	1.00 ±0.04	>150,000	245,000 + 35,000 - 25,000
38	Swanscombe	43.7 ± 0.4	0.24 ±0.04	1.48 ±0.03	1.31 ±0.05		1.44 0.06		
39	Swanscombe	67.3 ± 0.7	0.039 ±0.002	1.24 ±0.02	1.01 ±0.04	326,000 + 99,000 - 54,000	1.01 ±0.04	>164,000	>272,000
63	Swanscombe	42.6 ± 0.4	0.18 ±0.01	1.35 ±0.02	1.21 ±0.05				
41	Stutton	22.7 ± 0.2	0.048 ±0.002	1.21 ±0.02	0.808 ±0.032	165,000 ± 14,000	1.14 ±0.06		125,000§ ± 20,000
42	Brundon	58.8 ± 0.6	0.019 ±0.001	1.21 ±0.02	0.919 ±0.037	230,000 ± 30,000	1.17 ±0.06		174,000§ ± 30,000

*Experimentally measured activity ratios.

†Calculated using ^{230}Th half life of 75,200 yr.‡Calculated using ^{231}Pa half life of 32,500 yr.§Open-system date after Szabo and Rosholt⁶.

Two samples from Swanscombe could not be dated. In sample 38, both the $^{230}\text{Th}/^{234}\text{U}$ and $^{231}\text{Pa}/^{235}\text{U}$ ratios are anomalously high (1.31 ± 0.05 and 1.44 ± 0.66 , respectively). That is, the amounts of the daughter elements ^{230}Th and ^{231}Pa are in excess with respect to their radioactive parents, ^{234}U and ^{235}U . Accordingly, no ^{230}Th or ^{231}Pa dates can be calculated; and the open system model, which allows for the migration of uranium, also yields no finite date⁵. The $^{230}\text{Th}/^{234}\text{U}$ ratio of 1.21 ± 0.05 in sample 63 is also too high to yield a ^{230}Th date. Sample 39 from Swanscombe, on the other hand, has a measured $^{230}\text{Th}/^{234}\text{U}$ ratio of 1.01 ± 0.04 which yields a finite ^{230}Th date of $326,000 + 99,000 - 54,000$ yr BP. The $^{231}\text{Pa}/^{235}\text{U}$ ratio of 1.01 ± 0.04 indicates that this sample remained a closed system during at least the last 164,000 yr of its history. The large error of the ^{230}Th date arises because the calculated date is near the limit of resolution of the method.

Sample 41 from Stutton and sample 42 from Brundon yield finite ^{230}Th dates but the amount of ^{231}Pa in both cases is excessive with respect to the parent isotope ^{235}U . Using the open system model the dates of sample 41 from Stutton and sample 42 from Brundon work out at $125,000 \pm 20,000$ and $174,000 \pm 30,000$ yr BP, respectively.

The Clacton deposits, including artefacts, mammalia and mollusca, have been placed by most workers in the Hoxnian Interglaciation⁷⁻⁹ although Collins¹⁰ and Kerney¹¹ have argued for an early-middle Hoxnian age (zone II). Singer *et al.*¹² have, however, suggested that the gravels belong in the Anglian Glaciation. The Swanscombe skull level is also placed by most workers in the Hoxnian^{7,9,13}, though possibly very close to the end (zone IV or late III)^{10,11}.

The Stutton deposits, only some 8 km from the type site of the Ipswichian⁹, are usually placed in the Ipswichian⁷. If, however, a temperate interval existed during the glaciation between the Hoxnian and Ipswichian, the Stutton deposits could possibly be correlated with this interval. Similarly, the Brundon faunal level, only tenuously connected with the Ipswichian in the absence of palaeobotanical data, though clearly post-Hoxnian, may correlate with a possible temperate interval between the Hoxnian and Ipswichian.

Among these samples only sample 37 from Clacton gives a satisfactory closed system finite date, of 245,000 yr BP. This date agrees with a 'medium chronology' for the Pleistocene interglaciation. The date is too old for the short chronology of Emiliani¹⁴, and too young for the long chronology of Ericson *et al.*¹⁵. The Swanscombe date may be regarded as a minimum, at least 272,000 yr BP. Most chronologies would require Swanscombe to be later than Clacton; the circumstance can be

reconciled for the samples reported here by adopting the older limit for Clacton, that is about 280,000 yr BP.

The Brundon date, $174,000 \pm 30,000$ yr BP (open system), is older than most recent estimates for the last interglaciation, even the 120,000 yr age favoured by Shackleton¹⁶; it would correlate well with a Wolstonian temperate period (inter-Hoxnian-Ipswichian), or with the Ipswichian, if a longer chronology is assumed. The Stutton date, $125,000 \pm 20,000$ yr BP (open system), is very close to Shackleton's¹⁶ estimate for the Ipswichian. Within the limits of statistical error, the Stutton date would also agree with a date of about 100,000 yr BP for the Ipswichian. Alternatively, this date, like that from Brundon, would correlate with a hypothetical inter-Wolstonian temperate period at about 145,000 yr BP; this leaves the Ipswichian to be mainly younger than 100,000 yr BP, as has been widely suggested.

The Clacton date may be compared with two K-Ar dates that bear somewhat indirectly on the age of the Holstein (Mindel-Riss) Interglaciation in Europe: the KA 409 date¹⁷ from Monte Cavo, 20 km south-east of Rome, which dates leucite from a post-Flaminian (Mindel/Elster) eruption at 277,000 yr BP; and a Leilenkopf III date of $220,000 \pm 40,000$ yr BP on lava from an eruption some 36 km SSE of Bonn in the 'mittlere Mittel' terrace of the Rhine¹⁸, which is roughly contemporary¹⁹ with the beginning of the Holstein Interglaciation. The Clacton, Monte Cavo, and Leilenkopf dates are all consistent with a date of about 250,000 yr BP for the early part of the Holstein Interglaciation, if allowance is made for the full range of statistical error.

The four dates reported here support a medium chronology (see refs 10, 20 and 21) for the Pleistocene interglaciations. The dates do not support the short^{14,18} or long¹⁵ chronologies. A date of about 250,000 yr BP would seem to be the best estimate at present for the time of the Holstein Interglaciation.

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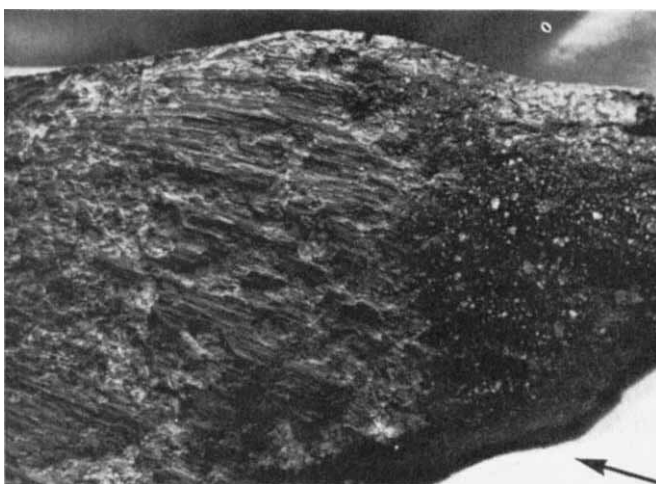
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Subglacial silica deposits

SUPERFICIAL carbonate deposits are common on bedrock surfaces recently exposed by retreating temperate glaciers^{1–5}. Their morphology indicates that they formed subglacially in intimate contact with sliding ice. Besides suggesting that chemical transport is an active subglacial process, these relatively widespread deposits reflect high solute concentrations in subglacial waters. This is a fact of considerable significance because solutes at the glacier–bed interface can impede significantly the sliding of temperate glaciers⁵. The dynamics of temperate glaciers and especially the more intriguing aspects of their behaviour, such as surging⁶, are thought to depend critically on the basal sliding process⁷. Thus, chemical exchange at the base of a glacier may affect significantly its entire motion. Moreover, because glacial erosion and deposition are largely dependent on glacial sliding, they too are affected by the presence of solutes at the bed. To date, reported subglacial deposits have all been carbonates. On the basis of previously unrecognised subglacial deposits rich in silica, I suggest that subglacial deposits are not exclusively carbonates, but that

Fig. 1 Glacially polished andesite surface on the right grades to the left into an irregular surface covered with a thin furrowed coating that formed in intimate contact with sliding temperate ice. Former direction of ice flow is indicated by the arrow, which is 2 cm long.



silicates are of wider significance, than is believed at present.

Figure 1 shows a sample of hypersthene–augite andesite, from the vicinity of Paradise Glacier in Mount Rainier National Park, Washington. This rock has a thoroughly striated and polished surface which merges in the down-glacier direction into a slightly irregular surface, coated with a thin light grey layer of mineral material. The surface of the coating is characterised by a pronounced lineation parallel to the local glacial striations, suggesting that it formed in intimate contact with actively sliding ice in much the same way as carbonate deposits⁵.

This millimetre-thick deposit consists of two contiguous, distinct types of material that differ markedly from the underlying bedrock. The more abundant type consists principally of angular, micrometre-sized rock and mineral fragments, presumably glacial flour, embedded in an amorphous matrix. The other type of material is finely laminated and contains only a few small clastic fragments. It ranges in colour from light to

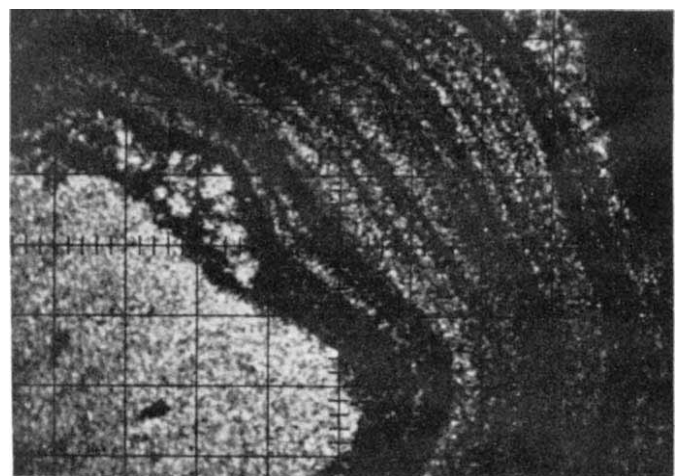


Fig. 2 Electron-probe, sodium-sensitive micrograph of feldspar phenocryst in andesite bedrock partially coated with finely laminated, subglacially precipitated, silica-rich coating. The lighter areas are relatively rich in sodium. Grid spacing, 25 μm.

dark brown in transmitted light and is characteristically optically isotropic, although in places it may be slightly birefringent. Electron microprobe analyses (EMA) indicate that the deposit is highly siliceous, in places consisting of as much as 95% SiO₂.

Figure 2 is an oscilloscope presentation of the characteristic X-ray radiation of sodium from an electron microprobe scan of the finely laminated, optically isotropic material partially coating a feldspar grain. The lamination is clearly compositional in nature with alternating layers relatively richer and poorer in sodium. A silicon micrograph of this same area revealed little structure in as much as the entire deposit is rich in SiO₂.

An X-ray powder diffraction pattern of the silica-rich coating showed sharp plagioclase peaks, presumably representing detrital feldspar grains in the deposit. There were no diffraction peaks of quartz or other silica polymorphs in the powder patterns. Transmission electron microscopic (TEM) examination of the deposit revealed a few submicrometre sized and larger, angular, mineral fragments embedded in a homogeneous matrix. No crystallographic subdomains could be detected in the matrix at magnifications of up to $\times 83,000$. Electron-diffraction patterns of the silica deposit invariably consisted of several families of spots, interpreted as a result of the presence of detrital grains of feldspar and ferromagnesian minerals. If the silica-rich matrix consists of crystalline subdomains, they are too small to cause significant diffraction of the electrons. Thus, the SiO₂ in the subglacially formed deposit is either

amorphous, or else the size of the ordered domains does not exceed several hundred angstroms.

Insight into the mode of formation of the subglacial silica-rich deposits can be obtained by analogy with previous studies of the subglacial precipitation of CaCO_3 , which is intimately related to the basal sliding of temperate glaciers¹⁻⁵. These glaciers, at their melting points throughout, flow by a combination of two processes: internal deformation and basal sliding. Sliding ice accommodates itself to irregularities in the bedrock by plastic deformation and by regelation. In the latter process, the basal ice melts in zones of higher pressure and reduced temperature, and the resulting water flows between the ice and bedrock toward zones of lower pressure and higher temperature, where it refreezes because of the pressure reduction. The heat necessary for the fusion is provided by the latent heat of freezing, which is conducted through ice and rock from the lee to the stoss sides of bed obstacles^{8,9}. The meltwater continuously produced by regelation sliding contains impurities originally present in the basal ice in addition to these resulting from the dissolution of the bedrock. As this water refreezes in areas of locally reduced pressure, generally along lee surfaces, solutes are continuously rejected into the liquid phase by the growing ice. The continuous rejection of dissolved silica, which is probably similar to the rejection of solutes in freezing NaCl (ref. 10), KOH (ref. 11), HCl (ref. 12), and CaCO_3 (ref. 5) solutions, and which must occur in the sliding process, will therefore cause progressive enrichment and eventual precipitation of silica from regelation waters in the lee of bed obstacles at the bases of actively sliding temperate glaciers. Solute concentrations are concentrated subglacially in much the same way as in the zone melting process used for refining metals and semiconductors; in this process impurities are removed preferentially by the passage of a molten zone in which impurities tend to accumulate.

Concentrations of dissolved silica as high as 8 p.p.m. are present in the water emerging from under two Mount Rainier glaciers¹³. Regelation melt water, flowing in intimate contact with the bedrock, should also contain high concentrations of dissolved silica. The subglacial, silica-rich deposits indicate that a portion of this mobilised silica is deposited, as an essentially amorphous precipitate trapping much glacial flour, at a temperature very close to 0 °C, a pressure not exceeding a few bars, and a pH close to 7 (ref. 13). The deposit I have mentioned (Fig. 1) formed recently, perhaps during the last major glacial advance, around 1850 AD (ref. 14).

Solute concentrations in regelation waters tend to inhibit glacial sliding because they accumulate at the lee of bed obstacles where freezing is localised. They reduce selectively the temperature there, thereby reducing the temperature gradient and thus the heat flow across bed obstacles that is essential for regelation sliding. Concentrations of silica exceeding the saturation value of 80 p.p.m. (ref. 15) can be expected where amorphous silica is precipitating subglacially. Together with other dissolved species generally present¹³, a difference in the concentration of solute of this magnitude between stoss and lee surfaces can be expected to inhibit glacial sliding significantly, especially if the glacier bed is characterised by uneven elements which have mean wavelengths that do not exceed 0.5 m (B.H., unpublished).

Subglacially formed carbonate deposits are relatively widespread, having been recognised on calcareous rocks in Scandinavia³, the Alps⁴, and the Rocky Mountains^{1,5}. This is the first report of subglacial silica-rich deposits. They are probably not restricted to the Mount Rainier locality, but rather, are likely to be widespread perhaps as thinner coatings and inconspicuous crack fillings, in glaciated areas underlain by silica-rich rocks. Considerable amounts of solutes are, therefore, likely to be present to the lee of bed obstacles at the base of practically all temperate glaciers, because most rocks are rich in carbonates or silica. Thus, the effect on glacial sliding is of general importance, because it will have a definite influence on the behaviour of practically all temperate glacial ice masses.

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Observations of a nonlinear interaction involving three electromagnetic waves in a laboratory magnetoplasma

POLARISATION requirements and wave number matching dictate that a nonlinear interaction involving three electromagnetic waves that propagate in a magnetoactive plasma must necessarily be two-dimensional if one of the waves propagates parallel to the external magnetic field $\mathbf{B}_0$. We report here what we believe to be the first observation of a three wave interaction of this kind. Our experiment involved the production of a whistler mode wave ($\mathbf{k}_3, \omega_3$) (referred to as WMW) through the interaction of two beams of high frequency electromagnetic waves ($\mathbf{k}_1, \omega_1$) and ($\mathbf{k}_2, \omega_2$), propagating somewhat above the local plasma frequency ω_p . The resonance conditions for the interaction are

$$\mathbf{k}_1 = \mathbf{k}_2 + \mathbf{k}_3 \text{ and } \omega_1 = \omega_2 + \omega_3 \quad (1)$$

If $\omega_{1,2} > \omega_p$ and ω_3 is sufficiently small compared with the electron gyrofrequency ω_c ($\omega_c < \omega_p$), the high frequency beams must be directed¹ almost at right angles to $\mathbf{B}_0$ (Fig. 1 inset). The strength of the coupling of the beams is determined by two factors: first, the magnitude of the matrix element for the interaction; and second, the time in which a stationary state is established as a result of the convection of the WMW packet across the finite interaction region of width, l . An expression^{2,3} for the steady-state amplitude, E_3 of the electric field of the WMW, can be obtained in terms of the amplitudes E_1 and E_2 of the beams

$$E_3 = \chi E_1 E_2 \quad (2)$$

where

$$\chi = \frac{1}{2} \frac{el}{mc^2} \frac{\omega_3^2}{\omega_1 \omega_2} \frac{\omega_c \mu^2}{(\mu^2 \omega_c^2 + 4\omega_1^2)^{1/2}}$$

where e and m are the charge and mass of the electron, respectively, c the speed of light, μ , the refractive index for the WMW, and other symbols are as defined previously.

The laboratory plasma is produced by transverse r.f. excitation of Argon gas in a 1-m long, 15-cm diameter, glass tube immersed in an axial magnetic field variable from 20 to 200 gauss, and uniform to within 2% over 60 cm length. This

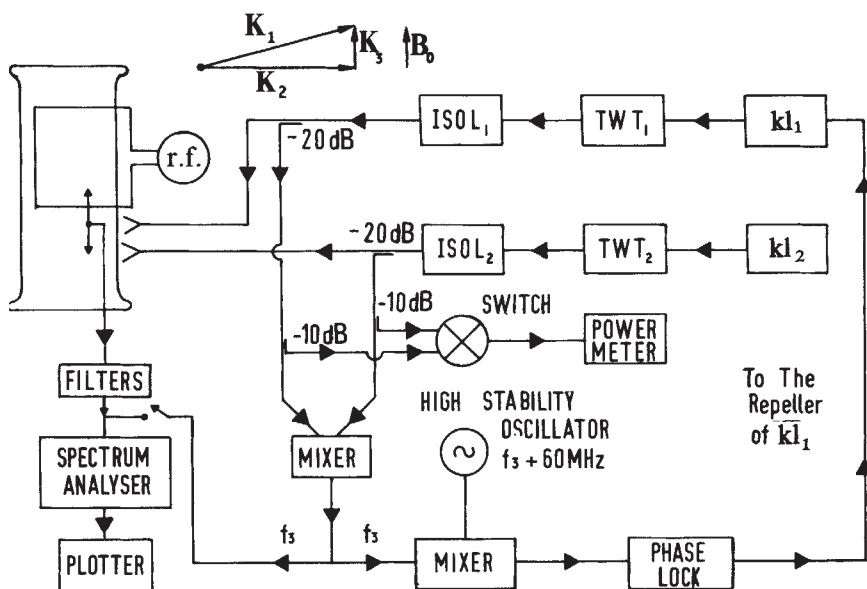


Fig. 1 Schematic diagram of the apparatus used in our experiment. For the true disposition and orientation of the horn antennae see text. Vector diagram at the top shows wave vector configuration for the interaction ($\mu_{1,2} \sim 1$, $\mu_3 \gg 1$).

method of plasma production^{4,5} creates a plasma with electron temperature $T_e \sim 3$ eV and density $n_e \sim 10^{12} \text{ cm}^{-3}$. Langmuir and wave field probes can be inserted radially into the plasma and a motor driven probe is used for the detection of the wave field along the tube axis.

Two microwave beams at frequencies f_1 and $f_2 \sim 11$ GHz are derived from two Klystron oscillator and TWT amplifier systems, and fed into the plasma through two horn antennae, which are situated side by side around the circumference of the tube. The beam from one horn (f_1) is oriented perpendicularly to the axial magnetic field with the electric vector E_1 at right angles to B_0 , and the other (f_2) is oriented at $5-10^\circ$ off the perpendicular, with E_2 parallel to B_0 . The power in each beam is controllable up to 15 W.

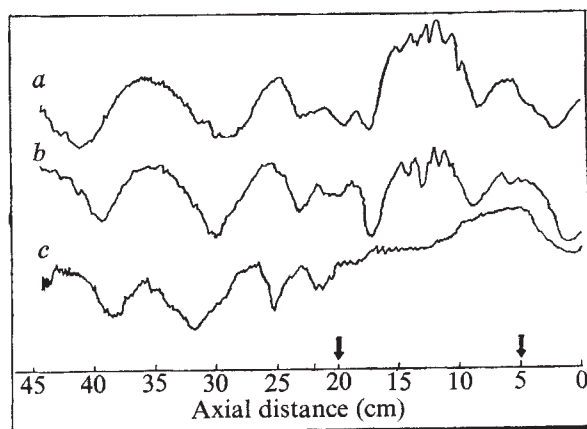
The frequency difference $f_3 = f_1 - f_2$ is stabilised using a double down converting system, locked to a 60 MHz standard which controls the repeller voltage of one of the Klystron oscillators, and can be varied at will in the range 0-400 MHz. As the deviation rate of the residual f.m. in the Klystrons is relatively slow, the response rate of the phase locked system is more than adequate to achieve overall frequency stabilisation to within some tens of Hz. Narrow band detection and frequency stabilisation are essential in the somewhat noisy plasma, and considerable care has been taken in eliminating pick-up.

Signals at f_3 are detected using the axial loop probe and a spectrum analyser (see Fig. 1). The combination of the frequency locking system and the stabilisation on the spectrum analyser results in an overall bandwidth at f_3 of ~ 20 Hz, with a sensitivity of -120 dBm, which corresponds to a minimum detectable oscillating magnetic field of 10^{-8} gauss for the frequencies we are interested in. Figure 2 shows the results of interferometric measurements of wave amplitude as a function of axial position of the probe, for $f_3 = 43$, 51 and 67 MHz, while the plasma conditions were kept constant. The two arrows on the figure define the length ($l \approx 15$ cm) of the interaction region, that is, the length of the plasma volume illuminated by the two microwave beams. At a short distance from this region the signal exhibits a clear phase variation with distance, from which the wavelength can be deduced. After allowing for small changes in wavelength ($\sim 10\%$) related to a weak beach field in the last 10 cm of the detection region, we have fitted (Fig. 3) the experimental points to the low frequency WMW dispersion relationship appropriate to the geometry of this experiment^{6,7}. The average

plasma density $n_e = 1.2 \times 10^{12} \text{ cm}^{-3}$, deduced from this fit, is in good agreement (to better than 20%) with the density deduced from Langmuir probe measurements.

The same figure also shows the dispersion relation for quasi-static waves⁸ which can exist in this experiment under the same plasma conditions. The identification of the waves at f_3 with a WMW is unambiguous. The existence of long wavelength, quasistatic waves in our apparatus was indeed established during preliminary investigations, by observing (in separate measurements without a reference signal in the spectrum analyser) the beating between short wavelength WMWs ($\lambda_s \sim 15$ cm) and quasistatic waves ($\lambda_{q.s.} \sim 200$ cm). We have also verified that the WMW field is proportional to the product of the input fields, and that for a fixed orientation of the high frequency beams, the amplitude of the WMW field varied with varying plasma parameters as predicted by the theory;

Fig. 2 Interferometer traces of WMW fields, detected by the loop probe, at three different frequencies, as a function of axial distance. a, $f_3 = 43$ MHz; b, $f_3 = 51$ MHz; c, $f_3 = 67$ MHz. Arrows on the distance scale mark the approximate extent of the region over which the microwave beams interact. The maximum amplitude corresponds to an electric field $E_e \sim 3 \times 10^{-4} \text{ V m}^{-1}$.



that is, it exhibits a broad resonance centred on the resonant condition of equation (1).

Care was taken to match the microwave beams at the glass-plasma interface, and microwave absorbers were packed around external sources of reflection. Nevertheless, some internal reflection did occur, and the possibility that regions of enhanced microwave field could give rise to other nonlinearities (for example, on the sheaths, probes, connections, and so on) and produce spurious signals at f_3 with the same spatial structure was investigated. These fields were examined by scattering measurements, and detection on the loop probe, and shown to be at least 30 dB down on the fields in the interaction region and to have a spatial structure along the axis totally unrelated to those obtained by phase measurements.

The magnitude of the coupling coefficient for the present experiment can be obtained by substituting the measured values of the amplitude of the high frequency beams ($E_1 \approx E_2 \approx 300 \text{ V m}^{-1}$), and the amplitude of the emerging WMW ($E_3 \sim 3 \times 10^{-4} \text{ V m}^{-1}$) into equation (2). This gives:

$$\chi_{\text{exp}} = 3 \times 10^{-9} \text{ m V}^{-1}.$$

On the other hand, the value of χ predicted theoretically from equation (3), using the experimental values for ω_1 , ω_2 , ω_p and ω_e gives

$$\chi_{\text{th}} \sim 1.3 \times 10^{-9} \text{ m V}^{-1}.$$

The good agreement between these two figures confirms the validity of the weak coupling model for this interaction.

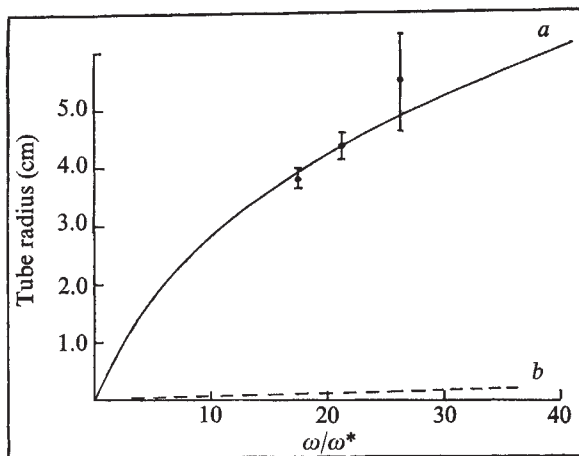


Fig. 3 Experimental data fitted to the theoretical dispersion curve (a) of low frequency whistlers (helicon waves⁶). $\omega^* = B_0/4\pi n e a^2$. The inferred value of the plasma frequency, n ($\sim 1.2 \times 10^{12} \text{ cm}^{-3}$) is in good agreement with independent Langmuir probe measurements⁷. b, Dispersion curve for quasistatic waves.

The original motivation for this experiment was to verify the theoretical predictions concerning the feasibility of an active field experiment¹ to excite WMWs in the Earth's ionosphere by means of two ground based high frequency transmitters of equal beam strengths ($E_{\text{ion}} = E_1 = E_2 = 1 \text{ V m}^{-1}$) and illuminating a region such that $l_{\text{ion}} = 2 \times 10^4 \text{ m}$.

It is interesting to extrapolate the laboratory measurements of χ to the ionospheric situation described here. This can be achieved by noting that the coupling coefficient for equal beam strengths scales as El (where $E = E_1 = E_2$), if the ratios

ω_3/ω_p , $\omega_{1,2}/\omega_p$ and ω_p/ω_e are kept constant. We then find that

$$\chi_{\text{ion}} = \chi_{\text{lab}} \left[\frac{(El)_{\text{ion}}}{(El)_{\text{lab}}} \right] \\ = 1.3 \times 10^{-6} \text{ m V}^{-1}$$

which is in good agreement with values calculated previously¹.

Finally, the positive results of this laboratory experiment seem to substantiate the suggestion that the very low frequency bands observed⁹ from the satellite Ariel 3 (and subsequently¹⁰, from Ariel 4) over the magnetically conjugate area of the eastern USA may be caused by the interaction in the Earth's ionosphere of powerful, medium wavelength signals (the f_1 and f_2 phases of our experiment) from commercial transmitters concentrated in that region, which excites WMWs (the f_3 of our experiment) which subsequently undergo natural amplification by a mechanism similar to that observed by Helliwell¹¹.

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Vacancy migration and void formation during annealing of electron irradiated molybdenum

WE have used a combination of three techniques—resistivity, positron annihilation and transmission electron microscopy (TEM)—to examine the recovery on annealing of point defect damage introduced into high purity molybdenum by 10 MeV electron irradiation. The work produced two points of particular interest, both arising directly from the sensitivity of the positron annihilation technique to vacancy defects.

The first point concerns the well known stage III recovery occurring in molybdenum at between 150 and 300 °C following either cold work^{1,2}, neutron irradiation^{3,4}, or electron irradiation^{5,6}. That this recovery stage is caused by the annihilation of vacancies with interstitials is little disputed but in molybdenum, as in the face centred cubic (f.c.c.) noble metals, there has been controversy as to the nature of the migrating species⁴. We therefore irradiated molybdenum specimens at 50 °C to a dose of $2.4 \times 10^{18} \text{ electrons cm}^{-2}$ and then combined liquid helium resistivity measurements with room temperature positron annihilation measurements to follow the changes occurring

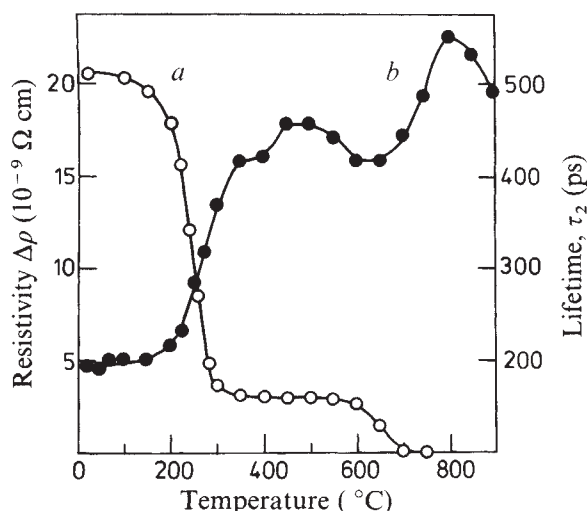


Fig. 1 The recovery of induced resistivity (a) and the behaviour of the long positron lifetime, τ_2 (b), during the isochronal annealing of electron irradiated molybdenum. Heating rate: 1°C min^{-1} .

during stepwise isochronal annealing (Fig. 1). The resistivity measurements showed that $\sim 80\%$ of the induced resistivity annealed out in stage III centred at $\sim 250^\circ\text{C}$ indicating, as expected, that the majority of radiation induced defects annihilate in this stage.

Meanwhile, in exactly the same temperature range the long positron lifetime increased from 195 ps to 400 ps. It is well established that positrons are sensitive to the presence of vacancies and vacancy clusters; the parameters of the clusters, such as shape and size, particularly influence the magnitude of $\tau_2^{7,8}$. On this basis, therefore, the significant rise in τ_2 can only be interpreted in terms of the formation of such clusters. Since it is difficult to see how interstitial migration can lead to this formation of vacancy clusters we believe that the stage III recovery in molybdenum must arise from the thermal migration of radiation induced vacancies. Models based on the migration of metastable interstitials or the detrapping of interstitials are, therefore, untenable; from the combination of resistivity and positron annihilation data it seems clear that during the vacancy migration a large number of the vacancies are annihilated at immobile interstitials (trapped at impurities during the irradiation^{6,9}) and that also, not unreasonably, a significant fraction of vacancies interact with each other to form clusters.

The second point of interest concerns the nature of the clusters, particularly as the 400 ps value for τ_2 suggested strongly that they could be three-dimensional. To obtain further experimental information we therefore continued annealing to higher temperatures. Figure 1 shows that the two significant stages are the well defined annealing of the remaining radiation induced resistivity at 650°C , and the 800°C peak in τ_2 where the long positron lifetime reaches a value of 550 ps before the lifetime parameters return to their pre-irradiation values. In the light of results from a previous analogous positron annihilation study on neutron irradiated molybdenum⁸ the interpretation of these two stages is comparatively straightforward. In the neutron work it was suggested⁸, and subsequently confirmed by TEM¹⁰, that a large rise in τ_2 in the range $750\text{--}900^\circ\text{C}$ was caused by the thermal coarsening of voids, whereas an earlier shoulder on this peak at $650\text{--}700^\circ\text{C}$ reflected the growth of voids at the expense of thermally shrinking vacancy loops.

Thus, it seems that during the stage III vacancy migration the vacancies escaping annihilation at interstitials nucleate clusters in two forms, one planar and one three-dimensional, which then grow to vacancy loops and voids, respectively. On annealing, the sensitivity of resistivity measurements to lattice

strain effects allows the thermal shrinkage of the small vacancy loops to be detected, leaving the void behaviour to be followed by the positron lifetime parameters. These conclusions are fully supported by investigations using the angular correlation technique (M.E. *et al.*, unpublished).

For further confirmation we cut disc-shaped specimens, suitable for electron microscopic study from the resistivity foils; these were annealed to 900°C before transmission microscopy examination at 100 keV. Small voids with diameters of $20\text{--}30\text{ \AA}$ were found within the foils; the void concentration was about 10^{13} voids cm^{-3} . Of the estimated number of vacancies (70 p.p.m.) surviving the irradiation, certainly not more than 1% survived the annealing to form voids.

It is worth emphasising the importance of these positron annihilation results because they reinforce the usefulness of such measurements in point defect studies. Furthermore, it seems possible that outstanding problems concerning other metals—for example, the vacancy–interstitial problem in stage III of the noble metals, or the intrinsic–extrinsic defect argument for stage III in the group V refractory metals—might be profitably examined by adding positron annihilation measurements to the normal range of techniques used in such studies.

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Fossil hominid body weight and brain size

RELIABLE estimates of body weights in early hominids are essential to the study of their brains and body proportions¹. Obviously the evolution of the size of the human brain must be studied in terms of the size of the body. Although the endocranial volume is known for many individual fossil hominids, body weight is poorly known. The present study is an attempt to estimate body weight of the South African Plio-Pleistocene hominids as accurately as possible from the available fossil evidence. East African early hominid postcrania are excluded because of the difficulty in taxonomic assessment, especially within the genus *Australopithecus*.

Most previous estimates of body weight for these fossils have been based on visual assessment or extrapolations from stature estimates^{1–5}. This study attempts to predict body weight directly from skeletal size. Of the fossils preserved in South Africa, the vertebrae present one of the best opportunities to do this since adult vertebrae of the same kind are known from both *A. africanus* (Sts 14 from Sterkfontein)⁶ and *A. robustus* (SK 3981 a and b from Swartkrans)^{5,6}. Also, vertebral size is highly correlated with body weight in humans. In my human sample, a measure of cross-sectional area of the last lumbar vertebra has a correlation of 0.72 with body weight. A measure of the volume of the last thoracic vertebral centrum has a correlation of 0.70.

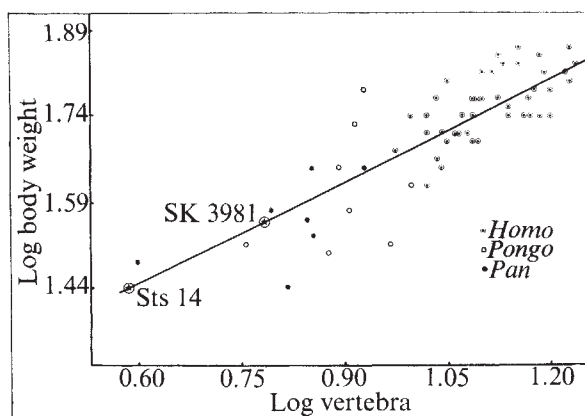


Fig. 1 Regression line and dispersion based on eight measurements of human vertebrae predicting body weight. Two male and five female *Pan* and eight female *Pongo* specimens are plotted for comparison purposes only.

The Terry collection at the Smithsonian Institution, Washington, provided the comparative human sample. This is one of the most extensive skeletal collections, with body weights at death, somatotype photographs, cause of death, sex, age, and other statistics. Forty-three specimens were used with about equal numbers from each sex. Excessively emaciated or obese individuals are eliminated as well as those over the age of 50 yr. For comparison several specimens of orangutan (*Pongo*) and chimpanzee (*Pan*) with known body weights are measured.

The sagittal and transverse diameters of the last thoracic and last lumbar centra are taken on the cranial surface and at the middle (corresponding to Martin measurements numbers 4, 6, 7 and 9)⁸. The average of the four sagittal diameters is multiplied by the average of the four transverse diameters forming an approximation of the cross-sectional area of the centrum. The regression formula:

$$\log(\text{weight}) = 0.603 \log(\text{vertebra}) + 1.085$$

has a correlation of 0.69 (Fig. 1).

Using this formula the body weight of Sts 14 is estimated to be 27.6 ± 10.5 kg (60.8 ± 23.0 lb) and that of SK 3981, 36.1 ± 10.7 kg (79.3 ± 23.5 lb).

These weights are probably close to the minimum for their taxa. The Sts 14 specimen (*A. africanus*) is one of the smallest known from Sterkfontein although its estimated body weight is similar to estimates made by other investigators using different methods¹⁻⁵. The estimate of body weight for the Swartkrans hominid (*A. robustus*) is also probably close to the minimum for that taxon but close to the predictions made by other authors¹⁻³. It is much smaller than the 68.1–90.8 kg (150–200 lb) estimate derived by Robinson⁵. The latter estimates seem excessively heavy since even using the great apes as models, the fossil remains are not consistent with such large weights. Figure 1 shows that *Pan* and *Pongo* vertebrae are close to the human regression line. Even some of the largest hominid postcranial remains from Swartkrans do not correspond to Robinson's minimum estimate. For example, of 14 *Pan* femora measured, the size of the SK 82 femur corresponds to an individual weighing 41.6 kg (91.5 lb). SK 97 is only slightly larger. If Robinson's estimates are correct, then the ratio of minimum posterior dentition surface area to body weight in *A. robustus* would only be about half that found in *A. africanus* which is just the reverse of what is expected^{4,9}. The other postcranial fossils of the South African *A. robustus* indicate a body size smaller than 150 lb (ref. 3).

If these body weight estimates are representative and reasonable minima, then the brain to body weight ratios can be calculated with more security than was possible before. There are several methods of comparing brain size and body weight. Using Holloway's¹⁰ estimates of endocranial volumes (442 cm^3

for *A. africanus* and 530 cm^3 for *A. robustus*), the encephalisation quotient¹¹ is 4.0 for *A. africanus* and 4.0 for *A. robustus* (compared with *Homo* = 7.1 and *Pan* = 2.5). The index of progression¹²⁻¹⁴ is 16.4 and 16.6 in the two fossil forms (*Homo* = 28.8 and *Pan* = 10.4). The constant of cephalisation^{12,15} yields values of 42.0 and 47.4 (*Homo* = 103.5 and *Pan* = 35.5). Finally the extra neurone index¹⁶ gives 3.9 billion and 4.4 billion (*Homo* = 8.5 and *Pan* = 3.6).

Figure 2 presents these indices of encephalisation as percentage deviations from *H. sapiens*. Although there is considerable debate about which of these indices allows the most meaningful comparisons¹¹⁻¹⁸, a general pattern emerges: the two forms of South African early hominids are similar to one another and intermediate between *H. sapiens* and *Pan* although much closer to the latter. These findings do not support the view expressed by Holloway¹⁹ that the australopithecine brain bears the same relationship to body weight as it does in modern humans. The relative size of the brain in these early hominids is very small. These fossil forms can be characterised as relatively small-brained but bipedal hominids, a grade in human evolution predicted in general by Darwin, and specifically by Haeckel, in which upright posture precedes brain expansion^{5,7,20,21}.

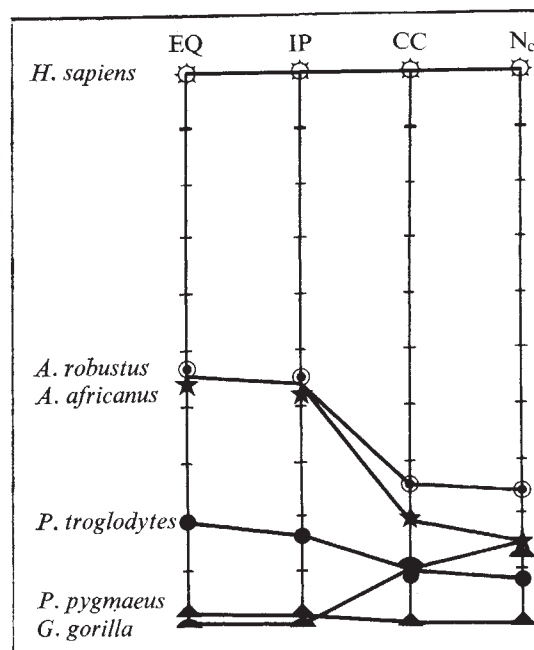


Fig. 2 Encephalisation indices expressed as percentage difference from *H. sapiens*. Comparative data derived from various sources¹¹⁻¹⁶. EQ, encephalisation quotient¹¹; IP, index of progression¹²⁻¹⁴; CC, constant of cephalisation^{12,15}; Nc, extra neurones¹⁶.

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How the cerebellum could memorise movements

THE neurones of the cerebellum are arranged in an extremely regular fashion and their synaptic connections are known in detail¹; this has led to considerable speculation about the functioning of these neuronal circuits. In particular, following a proposal that the cerebellum is involved in the learning of movements², there have been a number of theories about how the cerebellar circuitry could be used in this way³⁻⁵. These theories are all based on the postulate that the signals relating to motor output which are to be stored in the cerebellum have been computed in the cerebral cortex, and transmitted from there to the cerebellum for storage. They assume that the initial learning process, during which the animal learns to produce motor outputs with favourable consequences for itself, takes place somewhere in the cerebral cortex and not in the cerebellum: only after the cerebral cortex has learned how to generate these motor outputs is the information for their production stored in the cerebellum. Recent results suggest, however, that this postulate is incorrect, and that the cerebellum is directly involved in the learning of motor actions which have satisfactory consequences for the animal. To account for these results I describe a new way by which the cerebellum could be used for storing information relating to movements.

A previous theory⁵, which followed an earlier one about learning in single Purkyne cells³, showed how groups of Purkyne cells arranged as in Fig. 1 (called a unit) could be used for this information storage. The learning process was postulated to occur by changes in the strengths of the parallel fibre synapses on the Purkyne cells in a unit when the climbing fibres fired. It was demonstrated that a unit could learn to respond with a certain output (that is with a particular frequency of firing in the nuclear cell) to a certain parallel fibre input (that is a specific pattern of frequencies of firing in the parallel fibres). This output would then cause movement in a muscle and sensory feedback would create a new pattern of firing in the parallel fibres. If the unit had learned to respond to this new input then a new output would be produced and the movement would progress in this manner.

This method of memorising movements assumes, however, that the animal already knows how to perform the movements, and can send the appropriate signals required for a movement from the cerebral cortex, by way of the climbing fibres, to be stored in the cerebellum. An alternative scheme is suggested by work on the nucleus locus coeruleus. Thus it is known that axons from the noradrenaline-containing cells of the locus coeruleus contact Purkyne cells in the cerebellum⁶ (as shown in Fig. 1), and that stimulation of the locus coeruleus cells inhibits the firing of Purkyne cells⁷. Furthermore, the locus

coeruleus is connected with the reward, or positive reinforcement, system of the brain in that it will support intracranial self-stimulation behaviour⁸.

Therefore the locus coeruleus could be used in the consolidation of the motor signals stored in the cerebellum. This would be done in the following manner. When a movement was carried out the changes in synaptic strengths would be the same as those described above, but they would not be made permanent unless a signal was sent by means of the locus coeruleus axons to the Purkyne cells in a unit indicating that the outcome of the movement was satisfactory and that the movement was suitable for storage. This signal is presumably caused by increased rates of firing of locus coeruleus cells which then release larger quantities of noradrenaline at their synapses on the Purkyne cells. The release of noradrenaline has been shown to lead to the phosphorylation of membrane protein in guinea pig cerebral cortex⁹, and the requisite changes in strengths of parallel fibre synapses could possibly be mediated in this fashion in Purkyne cells. Although the immediate effect of noradrenaline is to inhibit the firing of Purkyne cells⁷, a mechanism like this could lead to a long term increase or decrease in synaptic strengths. Crow¹⁰ has proposed that the noradrenaline system could be used for transforming short term synaptic changes into long term changes. Because a motor action would be made up by a sequence of outputs from the units of Purkyne cells, there would probably be a signal requiring the synaptic changes corresponding to a number of these outputs to be made permanent simultaneously. As the synaptic changes at a parallel fibre synapse required for the storage of successive outputs are additive⁵, there is no difficulty in constructing biochemical models for this type of memory storage.

Figure 2 shows the different stages involved in the learning process. For long term memory, signals are required at a synapse from three separate sources. The initial short term

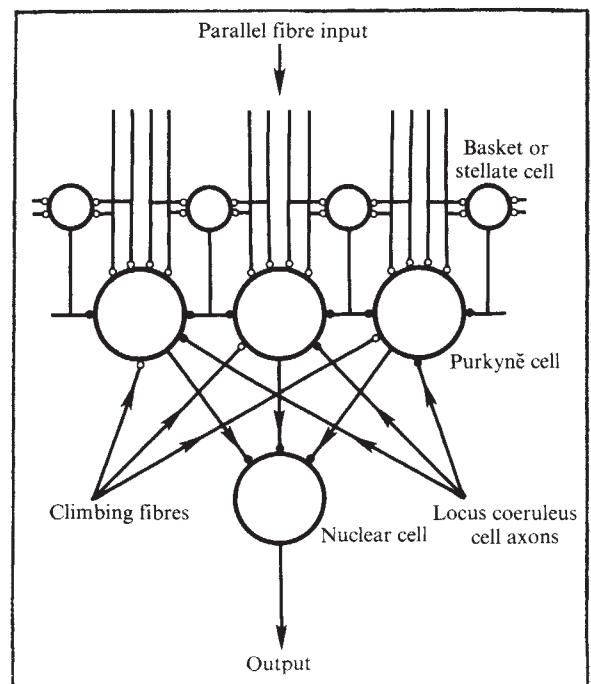


Fig. 1 The diagram shows the arrangement of the cells in one of the units involved in memorising movements. Each unit contains a large number of Purkyne cells which project to the same nuclear cell, or group of nuclear cells⁵. Every Purkyne cell receives excitatory inputs from as many as 200,000 parallel fibres, and inhibitory inputs from basket and stellate cells¹. The Purkyne cells are also contacted by climbing fibres and fibres from locus coeruleus cells. The unit can learn to respond to different parallel fibre inputs with specific frequencies of firing in the nuclear cell, through appropriate changes in the strengths of the parallel fibre synapses.

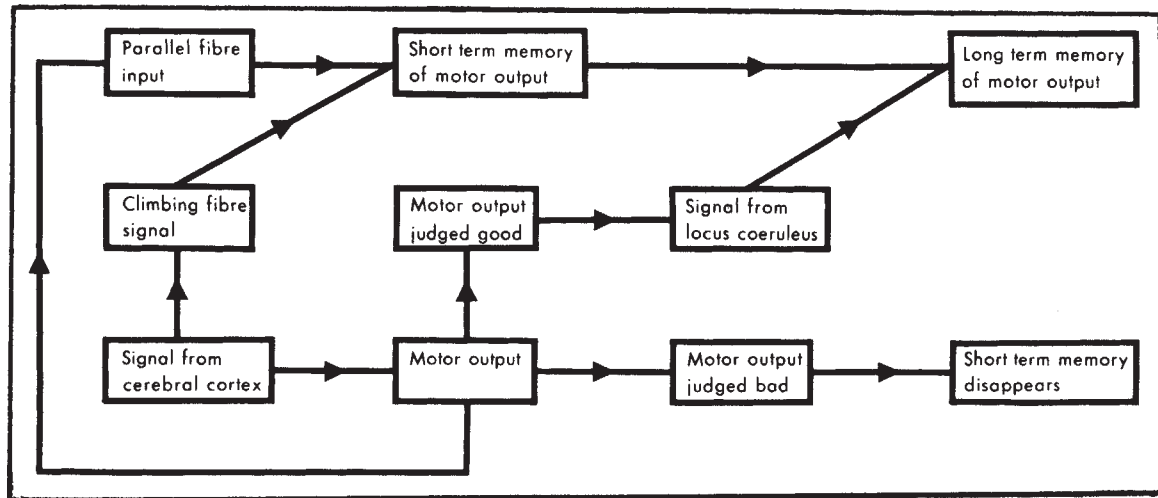


Fig. 2 The proposed scheme for the storage in the cerebellum of information relating to movements is shown. Initially a movement is carried out through signals from the cerebral cortex. These signals cause climbing fibre signals in the memorising units of the cerebellum projecting to the muscles involved in the movement. The simultaneous firing of the parallel and climbing fibres produce changes in the parallel fibre synapses on the Purkyne cells giving a short term memory of the movement. Only if the movement is judged to be satisfactory will a signal be sent from the locus coeruleus to convert the memory into a long term form.

changes in strength of synapses are produced in the cerebellum by the conjunction of signals from two of these sources transmitted by the parallel and climbing fibres. The synaptic changes take place at the parallel fibre synapse and it is the parallel fibre input to the cell which is used for the memory recall process. The climbing fibre input has a different role: it specifies the times at which motor outputs are to be memorised by a unit and later recalled during the learned movement. It was originally postulated⁸ that the climbing fibre could transmit signals of differing magnitude so that the unit could learn to fire with different output frequencies (this accounted for the observed changes in the frequency of firing of cerebellar nuclear cells during the performance of learned movements¹¹). Recent observations of climbing fibre signals during the learning of movements¹² do not, however, support this postulate. With the present memorising scheme it is only necessary for a climbing fibre to send signals of one magnitude and cause only one output frequency of firing to be learned by the unit (the memory capacity of a unit is very similar in this situation). If this frequency were not sufficient for the muscular action at a certain point in the movement then a second signal could be sent the next time the movement was practised, and this could be repeated until the movement was judged completely satisfactory. The changes in strength at the parallel fibre synapses are not made permanent and converted into a long term memory until a signal is received from the locus coeruleus. If the motor output is unsatisfactory and no signal is received then the short term changes in the synaptic strength will decay to zero and there will be no memory of this motor output. Presumably the spontaneous firing of climbing fibres about 1–2 times per second¹ causes short term changes of this type which are not consolidated into long term changes.

Evidence for this proposed role of the locus coeruleus in the learning of movements comes from a study¹³ on the effect of locus coeruleus lesions in rats. These lesions abolished the ability to learn to run with increased speed for a food reward, while causing no apparent motor deficit. Similarly it has been shown¹⁴ that depletion of brain catecholamines produces a severe impairment in the learning of a complex motor sequence, though once the task is learned there is no retention defect.

Various parts of the brain besides the cerebellum are involved in the production of movements, and therefore these studies show that the locus coeruleus may be involved in learning elsewhere in the nervous system. This is also indicated by the

widespread distribution of terminals from locus coeruleus cells to regions other than the cerebellum¹⁵.

In conclusion, the cerebellum seems to be a favourable system for studying the neuronal circuitry associated with short term and long term memory. The failure to demonstrate learning in the cerebellum at the cellular level¹⁶ could have resulted because no account was taken of the locus coeruleus in these experiments.

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Acceptance of novel flavours is increased after early experience of diverse tastes

WE believe that the more an animal experiences diversity of flavours at one time the more it will accept novel flavours later. Thus, rats given several distinctive flavours early in life would be more likely to accept an unfamiliar flavour later than would rats whose early gustatory experiences have been less varied. This hypothesis has had some empirical support in the literature. Kuo¹, for example, mentions pilot research suggesting that cats, dogs and mynah birds raised on restricted diets for

extended periods avoided new food, while others of their kind reared on varied diets ate novel foods readily. We have now found a similar effect with rats using flavoured solutions rather than solid foods.

The basic experimental plan (Table 1) was to expose mature and immature albino and hooded male rats (equally divided between ages and conditions) to either one or several flavours in the 'training' phase of the study. Thus, for mature and immature rats alike, each of three groups was restricted to a single flavoured water to drink with its food for the 12 d of training, while a fourth group received all three of these flavours, one at a time, during the same period. A further group of immature rats was given tap water during training. The appropriate solutions and Charles River chow were available throughout the study. After a 2-d interim of water following training, the rats were given a choice between tap water and a novel chocolate solution for 10 d. During these two-bottle tests, as during training, the rats were never without food and drink except for the time it took each morning to weigh the animals, record the liquid consumed (to nearest 0.5 g) and refill the bottles to a common level (each holding more than the rat could consume in 24 h). A series of identical tests, lasting 6 consecutive days, was conducted a month after the initial block of tests, the rats being given tap water during the interim. Individually caged, the rats were naive at the start of the experiment with respect to the training and testing flavours, which all consisted of 30 parts tap water to 1 part flavour by volume. (All flavours were of an imitation variety (Kroger brand), except for the test flavour which was a pure chocolate extract.) The solutions, given at room temperature were refreshed periodically. The right-left position of the test bottles was changed after each day's weighing and refilling.

The main results for each age category are given in Figs 1 and 2, which present the mean percentage of chocolate solution

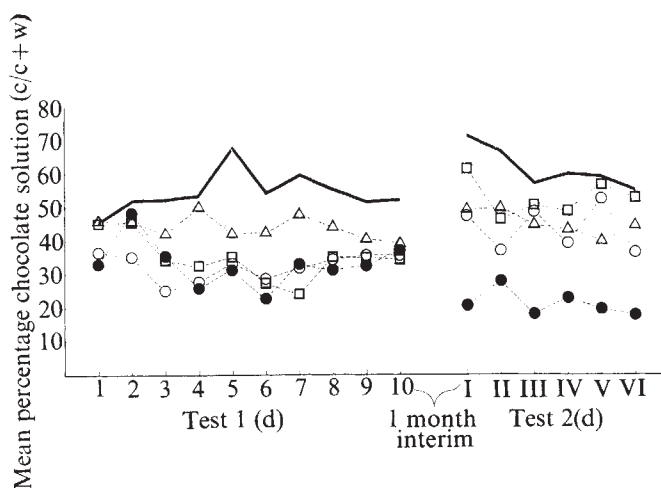


Fig. 1 Mean percentage chocolate solution consumed by immature rats (10 per group). Mean percentages of chocolate drunk over all test days: test 1, tap water (●) = 0.33; rum (□) = 0.35; black walnut (○) = 0.36; vanilla (△) = 0.44; varied (—) = 0.56; test 2, tap water = 0.21; black walnut = 0.44; vanilla = 0.45; rum = 0.53; varied = 0.62. (Symbols are the same for both tests.) Mean amount (g) of solution consumed in training (given first) and test 1: black walnut (23.7, 46.7); rum (21.7, 49.9), vanilla (25.7, 44.2); varied (26.8, 49.6); tap water (24.8, 39.8). The experiment was repeated with immature rats using maple rather than chocolate. A 30/1 solution of Kroger's imitation maple proved to be too palatable, with all of the groups, restricted and varied alike, drinking large proportions of the novel flavour. When the concentration of maple was increased to 10/1 midway through the first test series, the curves separated, with the varied group holding at a high level of maple consumption and all of the restricted groups declining noticeably for the duration of the tests. This illustrates the importance of using test flavours of only moderate to low 'natural' palatability so as not to obscure any training effects which might otherwise be there.

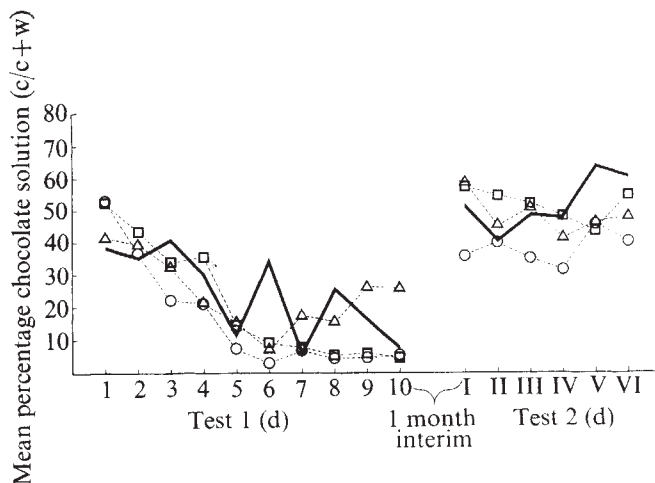


Fig. 2 Mean percentage chocolate consumed by mature rats (nine per group). Mean percentages of chocolate drunk over all test days: test 1, black walnut = 0.16; rum = 0.21; varied = 0.25; vanilla = 0.29; test 2, black walnut = 0.38; vanilla = 0.49; rum = 0.52; varied = 0.52. Mean amount (g) of solution consumed during training (given first) and test 1: black walnut (43.3, 51.8), rum (43.2, 49.5), vanilla (49.1, 46.7), and varied (49.8, 48.4). The symbols are as in Figure 1.

consumed in each of the initial tests (1–12) and in the second series (I–VI) given 1 month later. Individual scores were transformed (arc sin √ preference ratio) before statistical analysis to normalise the distribution. Basic to the design was our belief that relative consumption of the novel solution would be greatest by the 'varied group' trained on all three flavours. The prediction was borne out for the immature rats (Fig. 1): analysis of results for the first series of tests showing a significant training effect across training conditions ($F = 2.72$; d.f. = 4, 45; $P < 0.05$) but not over blocks of test days ($F = 1.33$; d.f. = 4, 180). A significant training effect allowed us to compare individual training conditions over all days in test 1 by a Newman-Keuls procedure². All restricted groups, including the water controls, drank significantly lower percentages of chocolate solution than did the rats trained on three flavours ($P < 0.05$). If a percentage of 0.50 or higher indicates a preference for chocolate, then only the varied group showed a slight to moderate preference for this novel flavour on several days of test 1. This is reflected in the overall averages reported at the bottom of Fig. 1, with the highest mean percentage of 0.56 in test 1 for rats trained on a variety of flavours. The intermediate mean of 0.44 for the vanilla group was significantly different ($P < 0.05$) from the water control (0.33), but not from levels reached by the other restricted groups (rum = 0.35 and black walnut = 0.36). This suggests that vanilla and chocolate taste more similar to rats than do any of the other training flavours and chocolate.

Results of the second series of tests for immature rats underscore the earlier finding, that is, relatively greater consumption of chocolate solution by the group that experience diversity of flavours early in life ($F = 3.39$; d.f. = 4, 45; $P < 0.05$). A significant main effect was found over blocks of test days ($F = 6.36$; d.f. = 2, 90; $P < 0.01$), with individual comparisons showing the varied group to have drunk significantly higher percentages of chocolate solution on days I–IV than its restricted counterparts ($P < 0.05$). In addition, all curves for the flavour-trained groups were well above the level reached by the water control rats for the entire test period ($P < 0.01$). This uniformly low consumption of chocolate in the control condition may reflect the impact of even a modest degree of diversity (be it a single training flavour) on later retention or augmentation of such preferences.

The results for the mature rats (Fig. 2) are unlike those for the immature subjects. Most importantly, these groups of rats trained at an older age did not differ from one another in test 1

($F = 1.22$; d.f. = 3,32), though they all showed, more or less, a steady decline in percentage of chocolate consumed over the 10-d test period ($F = 28.21$; d.f. = 4, 128; $P < 0.01$). When retested a month later, the chocolate consumption of all groups increased from that at the conclusion of test 1, approaching levels attained by rats trained when immature (comparison of test 2 in Figs 1 and 2). As in the initial tests, however, the mature groups did not differ from one another in test 2 ($F = 1.87$; d.f. = 3,32) in a manner consistent with our hypothesis. The mature rats, as a whole, more than doubled their mean percentage intake of chocolate solution from test 1 to test 2 (from 0.23 to 0.48), while the immature rats increased much less (from 0.41 in test 1 to 0.51 in test 2).

Finally, the average daily amounts of flavoured solution consumed by all groups during the training and first testing phases of the experiment are presented in the captions of Figs 1 and 2. For the immature rats, there seems to be a slight positive relationship between the amount of training flavour consumed and the percentage of chocolate drunk in testing, with the vanilla and varied groups ingesting the most chocolate and the most training solution. But although these two groups drank similar amounts during training, the varied group consumed proportionately more of the chocolate during both test periods than did the vanilla group.

Table 1 Summary of experimental design and procedure

Age (d)	(Immature)	25–36	37–38	39–48	49–79	80–85
	(Mature)	65–76	77–78	79–88	89–119	120–125
Group	<i>n</i>	Training	Interim	Test 1	Interim	Test 2
Restricted	10/9	Black walnut	T	24-h choice	T	24-h choice
			a		a	
Restricted	10/9	Rum	p	of novel†	p	of chocolate
Restricted	10/9	Vanilla	w	chocolate	w	solution or
			a		a	
Varied*	10/9	All three	t	solution or	t	tap water
			e		e	
Control†	10	Tap water	r	tap water	r	

*The flavour solutions for this group were varied every 2 training days, such that some rats received black walnut for the first 2 d, then 2 d each with rum and vanilla; others were given rum, vanilla and black walnut; and still others, vanilla, black walnut and rum, each order repeated for the duration of training. Each of the other groups was restricted to a single flavour (either black walnut, rum or vanilla) or tap water for the entire training period.

†This condition was included after the main study was completed. It serves as an additional comparison group (the most restricted of all) from which to gauge the effects of training. A comparable group of mature rats was not used because of the general ineffectiveness of training for these animals.

‡A 'novel' flavour is operationally defined here as one to which the animal had not been exposed before. Of course, it may well taste like something the rat had had, and this is the reason for the design used here. Since all the flavours experienced by the varied group are represented in separate experimental conditions, any differential effects found relating to the dimensions of diversity and restriction should reflect something more than a simple generalisation (or summation of generalisation) from training flavour(s) to test flavour(s).

Our findings show that young rats will accept an unfamiliar flavour more readily if their early taste experiences are varied rather than restricted. Furthermore, the effect seems to persist into maturity, at least for the conditions described here. A comparable effect was not found for rats who were older at the start of training. These rats did show, however, a dramatic decrease in chocolate consumption during test 1 and a subsequent (sustained) increase in test 2. Though we have no satisfactory explanation, a commonsense account of these results suggests simply that the older rats, treated as they were in this experiment, first tried chocolate, tired of it after a day or so, and then rebounded a month later because of their earlier (non-aversive) exposure to it. Obviously this is more description than explanation and additional research is needed before more can be said.

Our results suggest something different from what is

ordinarily implied by the concept 'stimulus' (that is, 'taste') generalisation, or the related concept of 'summation of generalisation'. This is we believe for two reasons: first, the effect was found only with immature rats (thus it is age-dependent); and second, none of the flavour-restricted immature rats, especially the water controls, reached the high levels of chocolate consumption shown by those trained on all three flavours—either during the immediate tests or a month later. In some sense, more flavours were familiar to these latter animals than to their more gustatorily sheltered counterparts. This is in keeping with an interpretation based on the notion of 'transfer of diversity', whereby the animal's earlier experiences (maybe at some 'sensitive' period in life) either do or do not prepare it for diversity or unfamiliarity later on^{3,5}.

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Independence of channels in colour contrast perception

IN the perception of brightness the responses from all three classes of cone in the human eye are pooled. Evidence from recent electrophysiological measurements¹, however, indicates that the contrast detected by a particular colour channel is assessed independently of the summation. Specifically, the existence of a red sensitive channel in which this occurred was demonstrated.

In the course of experiments concerned with the effectiveness of displays for use in aircraft cockpits^{2,3} we have compared the performance of subjects viewing red and green displays in a background of very high illuminance (10^5 lx). The results of these experiments, conducted in very different conditions at a much higher illuminance, support the suggestion of at least one visual colour channel in which contrast is assessed to some extent independently of other channels. These results also have important implications in the field of display design.

Each subject read two sets of 36 numbers and letters (each set forming the complete alphabet plus the numbers 0 to 9) presented singly in random order. The vertical dimension of the characters subtended 0.3° at the eye. A complete set in one colour was read before the colour was changed. Alternate subjects saw the colours in the reverse order and the results were averaged in order to eliminate learning or fatigue effects. The characters were back projected on to a small aperture in a screen in the high illuminance apparatus described previously². The screen is white in the upper half plane and black in the lower and thus simulates the conditions of an aircraft cockpit. Each character had a luminance of 100 cd m^{-2} and was presented for 0.7 s. Care was taken to balance the luminances of the red and green displays (peak wavelengths 660 and 560 nm respectively) and this was achieved to within 17%.

More than 120 subjects participated in the experiment, of whom 68 (55 male, 13 female) were free from visual defects. The results for these 68 subjects, expressed as an average error rate per subject are shown in Table 1 from which it is evident that the error rate for the green display was approximately

Table 1 Error rates for reading characters of different colours

	Error rate (%)	Omission rate (%)
Red display	5.8	1.1
Green display	15.9	5.6

Relative error rates for 68 observers (average age 31.5 yr) without visual defects, viewing red and green displays of equal luminance in an illuminance of 10^5 lx. The error rate includes characters wrongly identified and those for which no identification was offered (omissions).

3 times that for red. This finding is statistically significant at the 0.05% level.

Since the red and green displays were of equal luminance and the background illumination was the same for both, the contrast, considering total radiation, was the same for each display. Thus the large difference in error rates found in this experiment is consistent with the existence of one or more colour channels in which contrast is assessed independently of the assessment of the overall brightness.

We have estimated the ratio of the contrast in the red channel to that in the green channel although such calculations involve considerable uncertainties. Two approaches were used. For the first, we used the data of Wald⁴ for the action spectra of the red and green cone pigments. These data are supported by later results⁵ which give similar ratios (within 25%) of red to green cone response over the range of measurement (540–640 nm). Although the response to the red display was found to be exclusively associated with the red cones, the response to the green display was divided fairly evenly between the red and green cones (42:58). The effect of the background illumination was somewhat higher for the red cones (56:44). A comparison of the response of the red cones to the red display with that of the green cones to the green display, allowing for the different responses of these receptors to the background, showed the contrast in the red channel for the red display to be higher by a factor of 1.35.

The second estimate was based upon the high intensity colour mechanisms of Stiles⁶ and this calculation showed the contrast for the long wavelength mechanism π_5' when the red display was viewed to exceed that of the π_4' mechanism viewing the green display by a factor of 1.80.

Both of these estimates seem to be rather low to account for the large difference in error rates. They should be regarded as tentative, however, reflecting the difficulties of obtaining separate spectral responses for colour vision receptors as well as differing experimental conditions.

These considerations do not affect the conclusion that the large difference in error rates is consistent with the independent assessment of contrast in one or more colour channels and which therefore lends support to Regan's findings. Similar conclusions are emerging from response time experiments at present being conducted in this laboratory⁷.

A complete account of these and related experiments including details of the variation of performance with age, display format and character count, is in preparation. It is, however, worth mentioning that the consequences of the results given here for cockpit displays are of some significance. There is a popular belief that green displays are likely to be superior to red "because the eye response peaks in the green". Our results show that in very high ambient illumination this belief is ill founded and it is more difficult to make an effective green display.

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Inhibition and disinhibition of direction-specific mechanisms in human vision

MOTION-SENSITIVE mechanisms in human vision are selective for direction of stimulus movement. Psychophysical experiments reveal direction-specific channels which can be selectively desensitised or adapted^{1,2}. At threshold these direction-specific mechanisms operate independently; they have little or no sensitivity for the opposite direction of motion^{3–6}. Independence at threshold does not, of course, preclude interaction at supra-threshold stimulus levels. Indeed, inhibition has been found above threshold between otherwise independent spatial frequency-specific mechanisms^{7,8}, orientation-selective mechanisms^{9,10}, and binocular disparity-specific mechanisms¹¹. We report here comparable measurements of inhibition between direction-specific channels. We also show that in appropriate conditions the inhibition can itself be reduced or eliminated, a disinhibition effect.

To search for direction-specific inhibition at suprathreshold levels, we used a composite adaptation method^{7,8}. The term 'adaptation' refers only to the elevation of detection threshold produced by exposure to an appropriate high contrast stimulus; we make no assumptions as to the processes underlying adaptation^{12,13}. Contrast is defined as the peak-to-trough luminance amplitude of a grating divided by twice the average luminance. Stimuli were vertical sinusoidal gratings created on a cathode ray tube, using a television technique¹⁴ (10° square raster, average luminance 3.4 cd m⁻²).

Thresholds for a moving grating were measured following adaptation to either: a high-contrast grating (same spatial

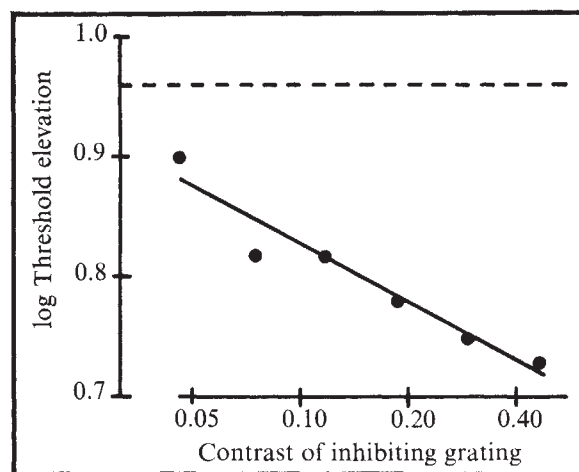


Fig. 1 Threshold elevation (1.75 cycles per degree, 7.9 Hz) for a rightward-moving test grating as a function of the contrast of a leftward-moving inhibiting grating. Contrast is specified separately for each component of the composite adaptation stimulus. Zero log threshold elevation represents the threshold for the rightward test grating after simple light adaptation (a zero contrast adaptation stimulus). The dashed line indicates threshold elevation produced by the rightward-adapting component alone. Each point is the mean of six measurements (s.e. < 6%). The slope of the solid line (fitted by least squares) is significantly less than zero ($P < 0.005$). Gratings were made to drift using motor-driven synchro-resolvers¹⁵. Viewing was monocular and foveal; fixation was carefully maintained. A session began with 3 min continuous exposure to an adaptation stimulus. Every 4 s thereafter the adaptation stimulus was replaced for 1 s by a test grating of variable contrast. The observer adjusted a potentiometer to reduce the contrast of the test grating until it was just indistinguishable from a uniform field of the same average luminance.

frequency and drift rate as the test) moving in the same direction as the test grating, or the same high-contrast grating to which had been linearly added another grating, moving in the opposite direction. An adapting stimulus which drifts in the same direction as a test grating (that is, the first type of adaptation stimulus) elevates test threshold; an adaptation grating which moves in the opposite direction (when presented alone) also elevates test threshold, although the amount of elevation is considerably less than is produced by a same-directional grating of equal contrast^{1,2}. One might expect these individual adapting effects to sum when a composite adaptation grating (the second type of adapting stimulus) is used. We have found, on the contrary, that addition of the opposite-directional grating gives less threshold elevation than does the same-directional grating alone. This constitutes an inhibitory effect.

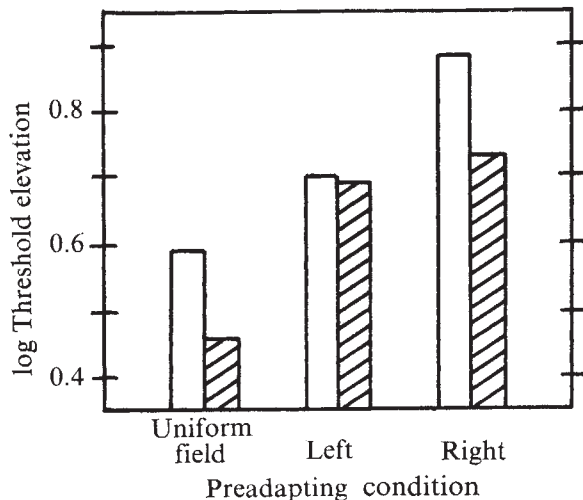


Fig. 2 Demonstration of disinhibition. Threshold elevation (1.75 cycles per degree, 7.9 Hz) for a rightward-moving test grating produced by adapting to rightward movement alone (open columns) or to rightward plus leftward movement (hatched columns) is shown separately for each preadapting stimulus. The bars are means of 12 measurements, s.e. < 6%.

Figure 1 shows the log threshold elevation for a test grating moving to the right as a function of the contrast of an inhibiting component (opposite direction) of the adaptation stimulus. The contrast of the adapting grating drifting to the right was fixed at 0.23. As the contrast of the opposite-directional component moving to the left increases, threshold elevation is reduced. The maximum inhibition effect is just over 0.2 log unit. Increasing the contrast of the component moving to the left reduces the adapting power of the grating moving to the right. The leftward grating seems to inhibit the activity of the rightward component. Similar data have been obtained at other spatial and temporal frequencies and with a second observer. The measurements have been verified under conditions of forced-choice testing as well.

These results show that direction-specific mechanisms can inhibit one another. We should also expect to see a related phenomenon, disinhibition, a reduction in the amount of generated inhibition when the inhibiting channel has itself been desensitised. Disinhibition is generally regarded as occurring when the original inhibition-generating channel is itself inhibited by a third channel. This is clearly impossible in the present experiment, since stimulus orientation must be held constant at vertical, and only two oppositely-tuned direction-specific channels may be considered. Disinhibition by adaptation of the inhibiting channel, however, should produce the same result, and can provide at least as much information about the inhibitory process.

To demonstrate disinhibition, we measured inhibition as above, but preadapted the inhibiting channel by exposure to its preferred direction of movement. For a test grating moving to

the right, the inhibiting component of the adapting stimulus moves leftward; the preadapting grating, then, must also move to the left. The direction-specificity of the adaptation process^{1,2} ensures that the preadapting stimulus will desensitise the leftward, inhibiting channel much more than the rightward, adapting channel. This should reduce the amount of inhibition exerted by the leftward mechanism on the rightward.

Each adaptation period was divided into two intervals, each of 1 s duration and continuously alternating throughout the experimental session. The first sort, a preadapting interval, was filled either with a leftward-moving preadaptation grating (contrast 0.46) or with an unmodulated field at the average luminance. During the second, the inhibition interval, the observer viewed either the rightward-moving adapting grating (contrast 0.23) or the same adapting stimulus plus the additional leftward-moving inhibition grating (contrast 0.46).

When the preadaptation stimulus was a uniform field (Fig. 2) a sizeable inhibition effect could be measured (about 0.13 log unit reduction in threshold elevation, $P < 0.001$, two-tailed t test). After preadaptation to the leftward-moving grating, however, no inhibition occurred ($P > 0.5$). To verify that this change did not result from the presence of any grating at all during the preadapting interval, we also preadapted to a rightward-moving (same-directional) grating (contrast 0.23), and obtained 0.16 log unit of inhibition ($P < 0.001$). Disinhibition seems to depend on preadaptation in the inhibiting direction (opposite to test direction).

This demonstration of disinhibition shows that the inhibition effect is not an artefact of nonlinear signal summation, either in the apparatus or in early stages of the visual system. Across all three types of preadaptation stimulus shown in Fig. 2, the configuration of the adapting and inhibiting gratings remained constant. The only changes were in the adaptive states of the observer's direction-specific mechanisms. Nevertheless, the inhibitory effect was eliminated in one condition. Thus, the inhibition effect cannot result from physical peculiarities of the composite stimulus.

Direction-specific neurones are known to be abundant in cat and monkey visual cortex¹⁶⁻²⁰. A direction-specific cell discharges strongly when stimulated by movement in one, preferred direction; the same cell fails to fire, or discharges at less than its maintained rate, when stimulated by movement in the opposite direction. Such reduction in a neurone's response to movement in its non-preferred direction can represent an active inhibitory process²¹⁻²³. The inhibition may be generated by other cortical cells, with different direction preferences²⁴. The present psychophysical demonstration of inhibition between human direction-specific mechanisms is thus compatible with the hypothesis that human direction-specificity is mediated by directionally selective neurones in human visual cortex.

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Genetic control of haploid parthenogenetic development in mammalian embryos

THE establishment of haploid cell lines in mammals would be of value for studies on developmental biology, genetics and carcinogenesis. It should be possible to produce such lines by culturing cells from haploid parthenogenetic embryos. Only a limited degree of haploid parthenogenetic development has been obtained with mammals^{1–4}, possibly as a result of the effects of deleterious recessive genes in haploid cells. During the process of selection necessary to produce an inbred strain of animals, there may be a reduction in the number of deleterious genes. Haploid embryos from random-bred

animals that have not been subjected to the selection that occurs in inbreeding may, therefore, have a lower development potential than haploid embryos from an inbred strain. To test this possibility, we have compared the development of haploid embryos from one inbred strain of golden hamsters with random-bred animals and have found better development of haploid embryos in the inbred animals.

We used an inbred strain (LSH/SS) and random-bred golden hamsters, because embryonic hamster cells can be readily transformed into cell lines by various viral, chemical and physical agents^{5,6} and their chromosomes can be easily identified. Parthenogenetic development has been induced in the mouse by electrical stimulation of the oviduct after superovulation with gonadotrophins^{3,4}. This technique also induces a high frequency of parthenogenetic development in hamsters.

The frequency of haploids and diploids was determined in parthenogenetically developing eggs at the single cell pronuclear stage, 10–12 h after electrical stimulation, and in embryos with more than two cells at 72–74 h after stimulation (Fig. 1). About 60% of the eggs were activated in both the inbred and random-bred hamsters at 10–12 h as determined by the presence of one or two pronuclei. At 72–74 h, about 30%

Table 1 Activation rate, egg development and haploid–diploid ratio, at 10–12 and 72–74 h after electrical stimulation of the oviducts of random-bred and inbred hamsters*

Time after electrical stimulation (h)	Random-bred or inbred animals	No. of females	Total no. of eggs examined	No. of embryos		% Activated eggs or embryos per animal†		% Non-activated eggs per animal†, ‡	Haploid–diploid ratio§
				Two-cell	More than two-cell	Cells with pronuclei	Embryos with two or more cells		
10–12	Random	5	96	0	0	57.7 ± 10.2	0	13.5 ± 4.4	3.2:1
10–12	Inbred	5	189	0	0	59.6 ± 9.0	0	7.2 ± 3.4	1.8:1
72–74	Random	26	725	107	65	0	25.5 ± 4.5	34.1 ± 5.4	0.2:1
72–74	Inbred	11	349	81	47	0	33.5 ± 4.4	24.4 ± 5.5	1.7:1

* Females (7–12 week old) from random-bred and the LSH/SS inbred strain of golden hamster, were superovulated by intraperitoneal injections of 25 IU of pregnant mare's serum gonadotrophin followed 48 h later by 20 IU of human chorionic gonadotrophin (HCG). Females were anaesthetised with an intraperitoneal injection of 0.3–0.4 ml of a 24 mg ml⁻¹ solution of Nembutal in physiological saline 19.5–21 h after the HCG injection.

Eggs were activated *in situ* by electric shocks (50 V), as described for mouse eggs^{3,4}. The tips of two steel needles, which served as electrodes, were applied along the length of the ampullar region, and 20–25 shocks applied in rapid succession. The animals were killed either 10–12 h or 72–74 h after treatment. Their oviducts were removed and flushed with phosphate-buffered saline to collect the eggs or embryos. Control groups without electrical stimulation were examined after superovulation and after Nembutal anaesthesia. About 20% of these eggs from the controls developed pronuclei, but no two-cell embryos were observed. When females were stimulated 15–16 h after HCG, only about 13% of the eggs isolated 72–74 h later were either two-cell or more advanced embryos. Higher rates of activation were obtained when the oviducts were electrically stimulated 19.5–21 h after HCG, so that this time was used in the experiments. Pronuclear eggs were cultured at 37 °C in NCTC 135 medium¹⁰ with 10% foetal calf serum, under light paraffin oil in plastic Petri dishes in an incubator with 5% CO₂ in air. The chromosomes of eggs were examined using an air-drying technique¹¹ in the first cleavage mitosis. As with mice^{12,13} all eggs which originally had a single pronucleus had a single haploid set of chromosomes, whereas those which originally had two pronuclei had a diploid first cleavage metaphase. Cleavage embryos (8–9) beyond the two-cell stage recovered at 72–74 h after electrical stimulation, were examined after culturing for 2–3 h at 37 °C in phosphate-buffered saline containing 4 µg ml⁻¹ colcemid. The chromosome ploidy and cell number of these embryos was then determined. The golden hamster has a normal diploid number of 44 chromosomes¹⁴. Some 70% of the embryos with mitotic figures had two or more metaphase plates.

† Eggs were only classified as activated if they had developed a pronucleus or had cleaved to the two-cell or later stage. The percentages of activated and non-activated eggs or embryos per animal are given as the mean ± s.e.

‡ In the 72–74 h groups there were two classes of eggs. Those which progressed to the first cleavage mitosis but failed to cleave to the two-cell stage, and those which were not activated. These two classes could not be distinguished. In all the experimental groups there were 30–40% degenerated eggs.

§ In the 10–12 h groups, the ratios were determined both on the number of pronuclei present, one or two, and on subsequent chromosome counts. The ratios in the 72–74 h groups were determined by chromosome counts on embryos that were more advanced than the two-cell stage. In the 72–74 h groups, the ploidy could not be determined in 50% of embryos from the random-bred and 37% of the inbred animals, because there were no dividing cells.

Table 2 Number of cells in embryos that had developed beyond the two-cell stage at 72–74 h

Random-bred or inbred animals	Haploid		No. of embryos	Diploid		Embryos without cells in mitosis	
	No. of embryos	No. of cells per embryo*		No. of cells per embryo*	No. of embryos	No. of cells per embryo*	
Random	4	7.0 ± 1.3 (4)	19	7.4 ± 1.1 (19)	23	7.1 ± 0.8	
Inbred	17	6.8 ± 0.7 (17)	10	10.7 ± 1.2 (10)	16	5.7 ± 0.8	

* Mean ± s.e.

Numbers in parentheses are numbers of embryos.

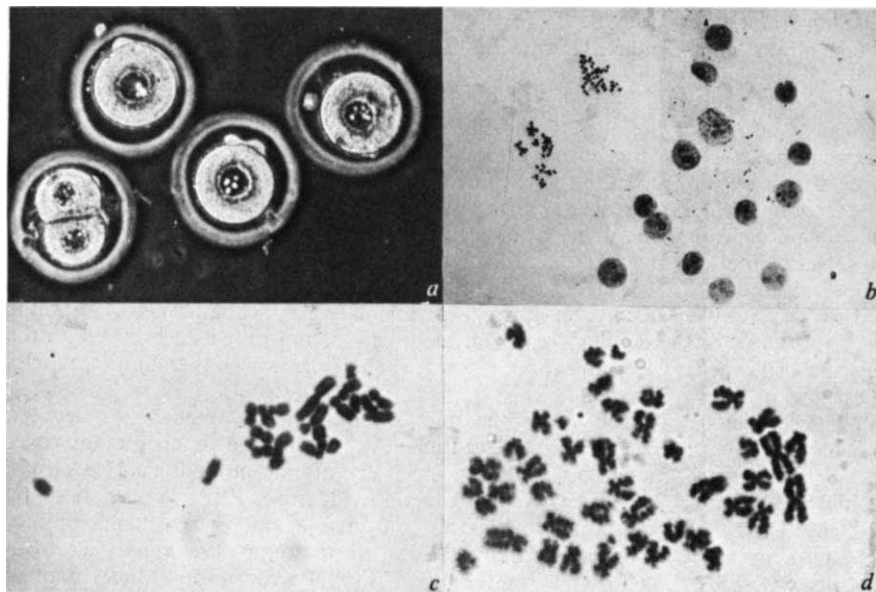


Fig. 1 *a*, Parthenogenetically activated eggs from a random-bred hamster: three single-cell eggs with one pronucleus and one embryo at the two-cell stage 16 h after electrical stimulation ($\times 350$). *b*, Diploid 16-cell parthenogenetic blastocyst from a random-bred hamster 72 h after electrical stimulation ($\times 250$). *c*, Haploid metaphase plate from an 11-cell parthenogenetic morula from a random-bred hamster 72 h after electrical stimulation ($\times 1,500$). *d*, Diploid metaphase plate from a 20-cell parthenogenetic blastocyst from an inbred hamster 72 h after electrical stimulation ($\times 2,500$).

of the embryos were either at the two-cell or a more advanced stage. At both times there were 30–40% degenerated eggs. Since the proportion of degenerated eggs was not higher at 72–74 than at 10–12 h, this suggests that 30% of the activated eggs found at 10–12 h after stimulation failed to develop to the two-cell stage in both groups of animals and were indistinguishable from non-activated eggs. Although in previous studies with golden hamsters parthenogenetic development did not occur beyond the two-cell stage^{7–9} we observed some well developed blastocysts with about 20 cells.

The ratios of haploids to diploids in the random-bred and inbred animals were 3.2:1 and 1.8:1 in the eggs at 10–12 h, and 0.2:1 and 1.7:1 in the embryos at 72–74 h, respectively (Table 1). The haploid to diploid ratio of eggs and embryos was, therefore, similar in inbred hamsters, with more haploids than diploids. In the random-bred animals, the ratio of haploid to diploid eggs was similar to that found in the inbred hamsters, but there was a much lower ratio of haploid to diploid embryos. The results indicate that in the random-bred animals, there was a block in more than 90% of the haploid eggs, so that they did not develop beyond the two-cell stage, but not in the inbred strain of hamsters (nor was there a block in two inbred strains of mice^{3,4}). The average number of cells in embryos which had developed beyond the two-cell stage at 72–74 h, was about the same in both haploids and diploids (Table 2).

These results indicate that the development of haploid parthenogenetic embryos can be genetically controlled. The better developmental potential of haploid embryos from the inbred compared to the random-bred animals presumably results from a reduction in the number of deleterious genes occurring during the process of selection associated with inbreeding.

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Polymer exclusion, cell adhesion and membrane fusion

FROM physicochemical studies on the adhesion of fibroblasts to synthetic substrata^{1,2} I suggested that, in general, surface-bound polymers (for example adsorbed serum proteins) probably inhibit cell attachment because of their steric exclusion volume. This effect is well known in colloid science (Faraday used gelatin to stabilise gold sols³) but does not seem to be widely appreciated in cell biology. Thus, a recent review on the role of proteins and divalent cations in cell adhesion, viewed as a problem in colloid stability, discusses only charge repulsion and calcium bridging⁴. Furthermore, standard texts on the cell surface⁵ present the classical DLVO (Derjaguin, Landau, Verwey and Overbeek) theory of charge repulsion against dispersive attraction, but omit the later development by Overbeek and coworkers of the principle that even uncharged surfaces, if covered by adsorbed polymer molecules, may repel each other⁶. I now show that steric exclusion by glycocalyx polymers on the plasma membrane would explain some recent observations on fusion and attachment, by a variety of cells. The term steric exclusion³ comprises not only geometric exclusion by rigid molecules, such as rods and spheres, but also unfavourable thermodynamic parameters such as free energy, summed up over many segments, and, for random coil polymers, entropy resulting from the ability of each molecule to claim extra lebensraum by flexing its segments.

This model predicts mutual repulsion between two closely apposed membranes, because of the exclusion volumes of their respective glycocalyx macromolecules. Conversely, fusion should be facilitated if glycopolymers are cleared from between the two membranes, by lateral displacement, to appose the naked lipid bilayers. Such a mechanism of fusion, by 'the interaction and mixing of the disturbed lipid molecules of closely apposed membranes in regions denuded of intramembranous proteins and glycoproteins' has been proposed⁷. The steric exclusion model, developed independently from studies on cell adhesion (S. P. Pegrum and N.G.M., unpublished), provides a general physicochemical basis for these observations on fusion. For instance, in discussing the

role of cryoprotective solvents in the lateral aggregation of intramembranous particles, Ahkong *et al.*⁷ concentrate on lipid effects, but exclusion implies interaction with the proteins of membranes. In terms of colloid chemistry, regarding the glycocalyx polymers as hydrophilic sols, such dehydrating solvents would be expected to cause their flocculation (for example, ref. 8). Moreover, the excluded volume for flexible polymers can be shown on general theoretical grounds⁹ to decrease in poor solvents, because of the increased preference for segment-segment contacts over segment-solvent interactions. (In fact the lateral aggregation of polymer molecules on a fluid interface may be a convenient method for the study of such interactions.)

A different aspect of cell adhesion which suggests the importance of avoiding fusion is based on the observation that cells do not adhere to pure, solid hydrocarbon¹. These experiments were originally carried out in serum, but have been extended to a protein-free medium², so that steric exclusion is not then a factor. In this medium cells attach and spread on condensed, rigid substrata with critical surface energy greater than 43 dyne cm⁻¹ (ref. 1) that is, those presenting pure amide, hydroxyl, ester and acid groups—so why not methyl and methylene? At first sight the inertness of hydrocarbon might seem to lack biological relevance—it is well known that solid paraffin is not a natural substratum for cell adhesion. This result seems remarkable if one recalls that cellular tissues contain high concentrations of almost pure hydrocarbon, both solid and liquid, near the front line of adhesion—in the lipid bilayer. I conclude that, contrary to the basic postulate of DLVO theories for cell adhesion⁵, the cell does not rely on non-polar, lipid-lipid interactions, because such interactions would lead to an increased probability of fusion between the hydrocarbon interiors of apposing lipid bilayers. Hence I postulate a model in which the mechanism of cell adhesion starts with two lines of defence against spontaneous and indiscriminate fusion of membranes—the first, steric exclusion by glycocalyx polymers; the second, reliance on polar, short range attractions rather than long range, non-polar dispersive forces.

Steric exclusion would also explain the low adhesivity of neutral, hydrophilic gels, such as agarose¹ or cross linked serum¹⁰. In a previous comparison of the supramolecular structures of agarose and collagen, I attributed the adhesivity of the latter both to the greater mechanical rigidity of its fibres and to their more substantial molecular packing¹. Subsequent experimental data has emphasised the importance of molecular aggregation. With or without serum, adhesion to rigid agarose is greatly improved if the latter is first annealed, near its setting temperature, to produce dense, granular microaggregates; this method of clustering double-helical agarose molecules in close side-by-side association was suggested by Dr D. A. Rees (N.G.M., unpublished). Similarly, Gordon and Dingle found that preincubation of soluble collagen, to form submicroscopic fibrils, increases its binding by platelets in serum-free buffer¹¹. I have found that increasing the concentration of a hydrophilic gel (sauflon polyvinylpyrrolidone; PVP) from 40% to 96% renders it adhesive for fibroblasts. These observations on the role of molecular clustering relate to preliminary reports, by Lyman and coworkers, that both thrombocytes and fibrocytes react to some microarchitectural or 'tacticity' difference between homologous copolymeric substrata¹².

They also suggest an answer to the problem of how cells overcome the exclusion barrier and adhere to polymer-coated surfaces. Divalent cations^{4,13} and bridge-forming 'adhesion factors'¹⁴ have been implicated, but longitudinal, that is, surface-to-surface, bridging does not explain one widespread feature of cell adhesion—the presence of dense, localised 'plaques'¹⁵ and desmosomes. These structures imply a role for lateral bridging as well. Cells fixed by cross-linking with aldehyde (and thus, I presume, with their membrane proteins intact but incapable of lateral movement) bind more weakly, whether to collagen directly¹¹ or to each other by way of a

bridging factor¹⁴. Lateral aggregation ('patching') is readily induced by lectin-like or antibody-like factors, in practice; but hitherto there has been no general reason to expect this as a feature of cell adhesion, in principle. The theory of excluded volume seems to provide a reason for the cell to concentrate glycocalyx polymers at the point of adhesion, because the excluded volume decreases as molecular crowding increases⁹. Since a large excluded volume depends on the possibility of molecules flexing⁶, the formation of dense lateral aggregates in the glycocalyx would restrict this effect, especially if matched by a corresponding clustering of the substratum (as in the above experiments on hydrophilic gels). Thus the cell may require divalent cations not only as a bridge with the substratum but also for the aggregation of membrane particles into plaques.

This raises the question of adhesive interactions between plaque and substratum. Although positive charge improves adhesivity¹⁰, the wide variety of non-physiological substrata, both negative² and neutral (agarose, PVP) implies bonding additional to that of fixed charge, on the one hand, or biochemically specific groups, on the other. Preliminary electron-microscope studies (with S. P. Pegrum) show direct contact and incipient fusion between membrane and substratum, in protein-free medium. These observations are more in agreement with Zisman's theory of adhesion as a process akin to wetting^{1,2} and thus additionally subject to close-range but nonspecific forces, such as hydrogen bonds and hydrophobic bonds.

I conclude that a model of cell adhesion must possess the following general features, which depend on physical chemistry, in addition to biological specificity of the sort found in antibodies, lectins and enzymes: minimal attraction between the hydrocarbon interiors of two apposing membrane bilayers; preference for solid, polar substrata; membrane glycopolymers inhibit adhesion and fusion through their exclusion volumes; exclusion effect countered by dense, local aggregates in glycocalyx (as in hydrophilic gels); this allows 'adhesion as wetting', that is, through short range but nonspecific bonds. A model cell based on these principles would be architecturally both stable (because the first three protect crowded membranes from spontaneous fusion and indiscriminate adhesion) and flexible (because the other two allow localised attachment to a variety of substrata).

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Possible involvement of the plasma membrane in saltatory particle movement in heliozoan axopods

THE suggestion that saltatory particle movements in heliozoan axopods, melanophores, nerve axons, as well as in other less specialised cells, are mediated by microtubules has been tendered¹⁻⁵. This notion is based on morphological observations, and experiments demonstrating the cessation or alteration

of movement in response to colchicine, a drug which destroys microtubules^{8,9}. Recent evidence, however, does not favour microtubules as the immediate driving force for such systems⁹⁻¹⁰.

Heliozoans are suited to studies of intracellular movement, because the kinetocysts¹¹ which shuttle back and forth within their axopods do so with a readily definable sign and path. The axopods contain a straight rigid core of microtubules^{11,12}. This report analyses the movement of kinetocysts in a heliozoan, and of bacteria, themselves non-motile, which occasionally adhere externally to the axopods. The results suggest that the plasma membrane, rather than the axonemal microtubules, may be involved.

Heterophrys sp., a small marine centrohelidian, was filmed at 10.25 frames per second. At five frame intervals the positions of continuously moving kinetocysts and bacteria were determined. The points obtained were tested for goodness of fit to a regression line¹³ of the form $y = a + bx$, where y represents particle displacement, a elevation, and x time. The regression coefficient b is then the best measure of particle velocity. To obtain enough points for an analysis of each displacement, only those movements which lasted at least 20 frames (1.8 s) were considered. The direction of movement, the distance traversed (d), and the approximate distance (D) of the particle from the cell body at the start of movement were also recorded.

Almost all of the displacements fitted a linear regression at the 5% significance level, indicating that, once in motion, the particles move with uniform velocity and thus their movements are clearly saltatory. The regression coefficients, however, ranged from 0.4 to 2.8 $\mu\text{m s}^{-1}$ ($n = 47$), demonstrating that the speed varies strongly between displacements. Since b for several displacements of the same particle also frequently varied significantly, the velocity of a given particle cannot be related simply to its mass or density.

Comparison of the average velocities of centripetally ($b_{in} = 1.09 \pm 0.11 \mu\text{m s}^{-1}$, $n = 25$) and centrifugally ($b_{out} = 0.64 \pm 0.05 \mu\text{m s}^{-1}$, $n = 13$) moving kinetocysts showed that these were significantly different ($0.01 < P < 0.05$). The Behrens-Fisher test was used because the variances were found to be significantly different in an F test ($P < 0.001$). A one-sided hypothesis permits the conclusion that kinetocysts move faster inward than outward ($P < 0.025$). A correlation test and a regression analysis showed that b_{in} and the distance moved

toward the cytosome (d_{in}) are significantly positively correlated ($r = 0.6516$, d.f. = 23, $P < 0.001$) and fit well the expression $d_{in} = -1.10 + 3.72 b_{in}$ ($P < 0.001$). Thus the faster the kinetocysts move toward the cell body, the longer are the distances traversed. No such correlation could be shown for centrifugally moving kinetocysts ($r = -0.4216$, d.f. = 11, $P > 0.1$). Additional correlation tests demonstrated that b_{in} and b_{out} and d_{in} and d_{out} , are not significantly correlated with D . Therefore, the velocity and path lengths of moving kinetocysts are independent of their position along the axopod at the start of saltation. Finally, tests of the null hypotheses, $H_0: d_{in} = d_{out}$ and $H_0: D_{in} = D_{out}$, were not significant. This supports the experimental technique, because they do not demonstrate a bias in the selection of inwardly and outwardly saltating particles with respect to their average path length or initial distance from the cytosome.

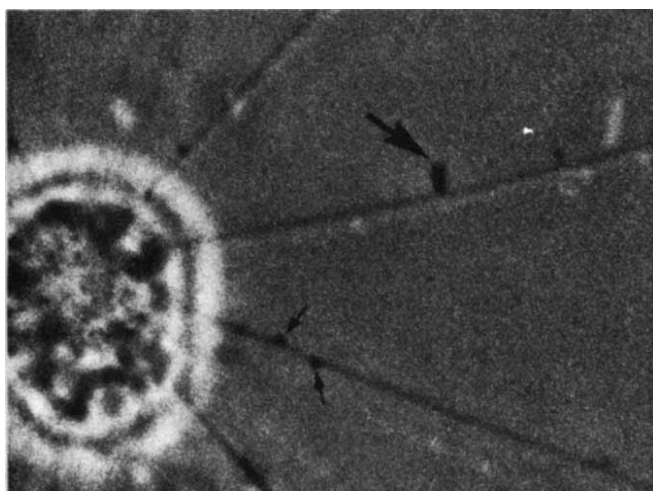
Although the data obtained for saltating bacteria were few, a significant correlation of b_{in} with d_{in} could be demonstrated ($r = 0.8890$, d.f. = 3, $0.02 < P < 0.05$). The expression for the regression of path length on velocity was $d_{in} = -1.08 + 4.42b_{in}$ ($P < 0.05$). A correlation test of b_{out} and d_{out} was not significant ($r = 0.9019$, d.f. = 2, $P > 0.1$). Thus the kinetocyst and bacteria saltatory movements seem to be similar in character, both showing correlation of b_{in} with d_{in} , but not of b_{out} with d_{out} . The regressions of d_{in} on b_{in} for kinetocysts and bacteria were compared in an analysis of covariance¹³. No significant differences between their residual variances ($F = 0.72$, d.f. = 23, 3), slopes ($F = 0.22$, d.f. = 1, 26) or elevations ($F = 0.70$, d.f. = 1, 27) were found, showing that the regression lines are not significantly different.

The different average velocities and variances of centripetally and centrifugally moving kinetocysts, and the correlation of velocity with path length during inward, but not outward movements of both kinetocysts and bacteria, imply that two fundamentally different mechanisms are responsible for transport to and from the cell body. A similar situation exists in melanophores where aggregation and dispersion are known to be differentially sensitive to drugs^{14,15}. The correlation of b_{in} with d_{in} also suggests that centripetal movement in axopods is an ordered process with elastic properties reminiscent of the snapping of a rubber band; the more the band is stretched before it is released, the faster and farther something attached to it will be moved. Centrifugal movement, in contrast, is unordered and wholly inelastic.

The microtubules of the axoneme can hardly be involved in saltation, for when they are destroyed by colchicine, similar movement continues, although not in straight lines (ref. 16 and D.T., unpublished). Since the kinetocyst and bacteria centripetal movements are statistically indistinguishable, it is possible that the forces motivating both are the same. The kinetocysts, however, move under the plasma membrane, whereas the bacteria are clearly extracellular, exhibiting unmistakable Brownian motion in the surrounding medium, especially when adherent at only one end (Fig. 1). If both types of particle are moved by the same mechanism, the possibility cannot be ignored that the probable site of force application is the plasma membrane itself, the only structure in common contact with kinetocysts and bacteria. Speculation that the membrane is involved receives support from a recent demonstration that the rate of area change and initial area of a triangle of plasma membrane delimited by three extracellularly adherent gold particles are correlated when the particles aggregate, but not when they disaggregate¹⁷. In other words, aggregating particles on the membrane move with precisely the same elastic qualities as shown here for centripetally saltating structures; particle disaggregation, like centrifugal saltation, is unordered.

Whether movement in axopods involves gradual autonomous stretching and subsequent elastic release of the membrane itself, or an interaction between membrane islands¹⁷ or anchorages¹⁸ and extrinsic contractile protein remains to be seen. That actin¹⁹ and myosin²⁰ exist in other cells in an appropriate position to be capable of causing the kind of membrane-

Fig. 1 Frame from a film of *Heterophrys* sp. A bacterium (large arrow) adheres at its end to an axopod. The small arrows indicate kinetocysts. Phase contrast, $\times 3,400$.



associated movement described here has already been shown. *Heterophrys* sp. was provided by Dr M. Hauser.

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Tryptic destruction of aggregative competence in *Dictyostelium discoideum* and subsequent recovery

WHEN they reach the stationary phase, the vegetative cells of *Dictyostelium discoideum* become adhesive and form multicellular aggregates before forming fruiting bodies. In shaken liquid suspension they form aggregates in two stages¹. First,

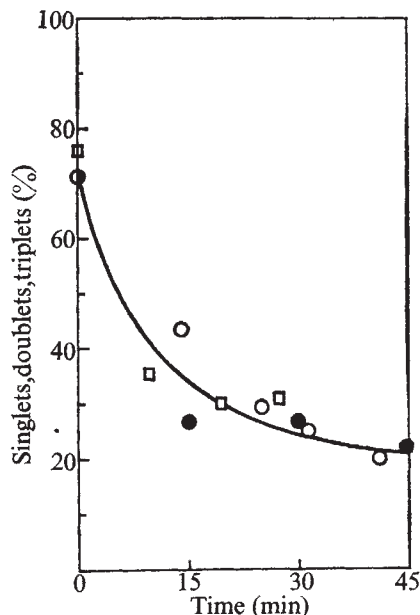


Fig. 1 Aggregates at the 15 h stage of fruiting body construction were harvested from filters in 17 mM K/Na₂ phosphate, pH 6.8, mechanically dispersed to a cell suspension by repeated forceful pipettings through a 10 ml pipette, and diluted to a density of 10^7 cells ml^{-1} in phosphate buffer containing the following additions: $\circ$, no additions, $\bullet$, 2% v/v glycerol, 10 mM EDTA, 0.5 mg ml^{-1} type III trypsin (T, Sigma), 0.5 mg ml^{-1} type I S trypsin inhibitor (T I, Sigma); $\square$, all of the previous additions plus 0.5 mg ml^{-1} cycloheximide (Sigma). Volumes (20 ml) of suspension in 150 ml Erlenmeyer flasks were incubated at 22 °C on a rotary shaker at 110 r.p.m. At intervals samples were examined in a haemocytometer to determine the distribution of cells as either singlets, doublets, triplets or larger aggregates. A significant reduction in the first category was accompanied by an equivalent increase in the second.

they produce loose amorphous clusters which, if deposited on a solid substratum, immediately disperse. In the second stage (6-7 h) the clusters have become tight, round or ovoid aggregates eventually covered with a thin cortical sheath. If deposited on solid substratum, they remain intact and immediately proceed to the next stage of fruiting body construction. The loose clusters are EDTA-sensitive, the tight aggregates are not².

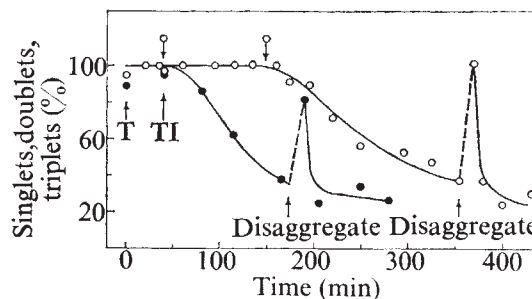
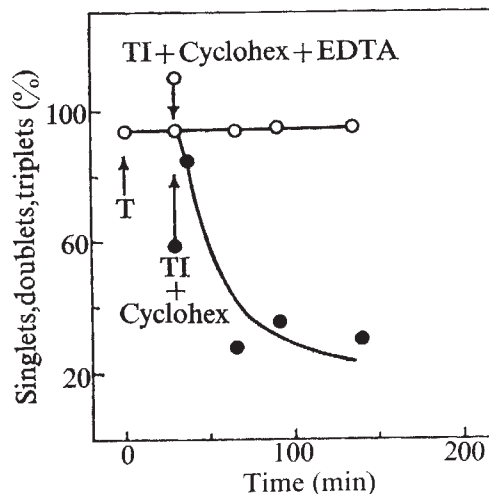


Fig. 2 Cells were collected at 15 h culture, dispersed and diluted to a density of 10^7 cells ml^{-1} in phosphate buffer containing glycerol, EDTA, and trypsin. After 40 min, trypsin inhibitor was added with ($\circ$) and without ($\bullet$) cycloheximide (first arrow). Cycloheximide was removed (second arrow) from the former by centrifuging the suspension for 10 min at 400g in a Sorvall swinging bucket rotor, washing twice in phosphate buffer containing glycerol and EDTA and resuspending the cells in 20 ml of that solution. The incubation was then resumed. When noted, aggregates were dispersed by repeated pipettings, as before to measure the kinetics of reaggregation. Concentrations as in Fig. 1.

Aggregated cells can be mechanically dispersed to yield a single cell suspension which if shaken gently reforms tight aggregates within a few minutes. The experiments to be described demonstrate that, first treatment of disaggregated cells with trypsin destroys their capacity to form tight EDTA-resistant aggregates without affecting their capacity to adhere in loose, EDTA-sensitive clusters; second, on continued incubation after removal of the trypsin by inactivation, the cells slowly reacquire aggregative competence but cannot do so in the presence of cycloheximide; and third, the initial aggregative competence, its destruction after trypsin treatment, and its subsequent reacquisition are precisely reflected in the adhesive properties of ghosts derived from the untreated and treated cells.

Figure 1 shows the kinetics of reaggregation by freshly disaggregated cells. Confirming the earlier finding², the process is EDTA-resistant. It is unaffected by trypsin already inactivated

Fig. 3 Trypsin treatment was carried out as before but in the absence of EDTA. At 10 min intervals the reclustered cells were redispersed by repeated pipettings. After 30 min, trypsin inhibitor and cycloheximide (cyclohex) were added with ($\circ$) and without ($\bullet$) EDTA. Concentrations as in Fig. 1.



by trypsin inhibitor, by glycerol (present to provide osmotic protection against trypsin) and by cycloheximide. In contrast cells treated with trypsin for 40 min before the addition of trypsin inhibitor reaggregated very slowly (Fig. 2) but after about 2.5 h had regained full EDTA-resistant, aggregative competence. The presence of cycloheximide prevented this reacquisition but the inhibition was shown to be reversible after removal of the drug. Clonal platings demonstrated that cell viability was unaffected by any of the treatments employed.

Disaggregated cells treated with trypsin in the absence of EDTA continually reclustered and had to be kept apart by frequent mechanical disruption. If after the trypsin was inactivated, EDTA (and cycloheximide) were added, the cells remained dispersed (Fig. 3). In the absence of EDTA, however, loose clustering was immediately resumed. Thus, trypsin treatment destroys only the second-stage adhesive capability and does not affect the first.

Recently it was demonstrated that ghosts derived from pre- and postaggregative cells retain the adhesive behaviour of the

cells from which they were derived. A cofactor requirement for aggregation could be satisfied by addition of any of a specific group of divalent cations (Mn^{2+} , Ca^{2+} , Zn^{2+} and Cu^{2+})³. Figure 4 shows that in the presence of Ca^{2+} and EDTA, ghosts prepared from disaggregated cells, from trypsin-treated cells and from cells allowed to recover from trypsin treatment in the presence and absence of cycloheximide reflected precisely the adhesive capacities of the cells from which they were derived. This provides assurance that the trypsin-mediated lesion is located in the plasma membrane and is intimately associated with the specific adhesive sites. We are attempting to determine what is removed from the membrane by trypsin treatment and what is replaced during the recovery period. It will also be interesting to determine the relationship, if any, between these tryptic lesions and the antigenic determinants on the membrane which accumulate during the preaggregative period and which have been implicated in the adhesive process^{4,5}.

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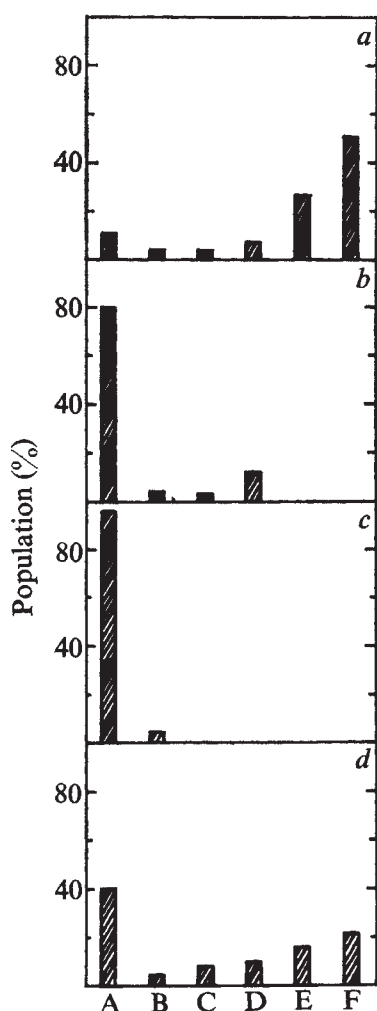


Fig. 4 Untreated control cells (a), cells treated with trypsin for 40 min (b), and cells allowed to recover after addition of trypsin inhibitor for 2.5 h with (c) and without (d) cycloheximide were collected, suspended at a density of 10^8 cells ml^{-1} in 5 ml aliquots of solution containing 20 mM K/Na_2 phosphate, pH 6.5, 50 mM NaCl, and 2% glycerol, and frozen at $-20^\circ C$. After thawing, the disrupted cells, now ghosts, were washed three times in phosphate buffer containing 1 mM $CaCl_2$ and 10 mM EDTA and resuspended therein at a density of about 10^7 ghosts ml^{-1} . Aliquots (1 ml) in tubes were incubated at $22^\circ C$ on a shaker. After 45 min, the population was examined microscopically to determine the distribution in groups of 1-3 (A), 4-5 (B), 6-10 (C), 11-15 (D), 16-50 (E), 51-100 or more (F).

Carbohydrate-mediated elimination of avian plasma glycoprotein in mammals

THE heavily glycosylated¹ plasma protein α_1 -acid glycoprotein (α_1 AGP) is known to occur in numerous mammalian species². Its biological significance is still largely unknown³ but together with fibrinogen and caeruloplasmin it belongs to a particular group of plasma proteins (acute phase reactants)⁴, the synthesis of which is a response to trauma.

We isolated from chicken serum an α_1 -acid glycoprotein which resisted precipitation at pH 3.0 and $2^\circ C$ with a combination of ammonium sulphate and trichloroacetic acid at the respective final concentrations of 2.18 and 0.075 M. Further purification of this protein was accomplished by ion exchange chromatography on Amberlite IRC-50 resin⁵. The product migrated with the mobility of human α_1 AGP as a single band when electrophoresed in 7.5% polyacrylamide gel at pH 8.3. Its molecular weight, as determined by ultracentrifugation using a low speed equilibrium technique⁶, was about 45,000 and it contained 44% (w/w) total carbohydrate (12.5% sialic acid⁷, 16.0% hexosamine⁸ and 16.0% neutral sugars⁹). Judged by these criteria this avian protein compares well with, and is therefore assumed analogous with, human α_1 AGP.

Human α_1 AGP and chicken α_1 AGP were labelled¹⁰ with different isotopes of iodine and injected simultaneously into adult chickens. The plasma protein-bound radioactivity curves closely paralleled each other, indicating no major difference in the catabolic rates of these proteins in the chicken (Fig. 1). By sharp contrast, when the same preparations were injected into New Zealand White rabbits the human protein underwent catabolism at rates which closely resembled rabbit α_1 AGP,

Table 1 Means $\pm$ s. d. for the recovery of various iodine-labelled glycoproteins with the rat liver

Experiment		Marker protein		Test protein		Treatment*
Code	No.	Type	Dose in liver (%)	Type	Dose in liver (%)	
A ₁	7	H α_1 AGP	12.7 $\pm$ 4.7	CH α_1 AGP	57.6 $\pm$ 8.7	None
A ₂	3	—	—	CH α_1 AGP	61.4 $\pm$ 4.3	None
A ₃	3	—	—	CH α_1 AGP	60.4 $\pm$ 1.5	None
B	3	H α_1 AGP	10.4 $\pm$ 2.7	CH α_1 AGP	57.8 $\pm$ 5.6	R α_1 AGP (2.4 mg per 100 g)
C	4	H α_1 AGP	12.9 $\pm$ 1.2	CH α_1 AGP	35.6 $\pm$ 4.9	H α_1 AGP (3.1 mg per 100 g)
D	4	H α_1 AGP	7.4 $\pm$ 1.2	CH α_1 AGP	8.4 $\pm$ 1.2	HAs α_1 AGP (3.0 mg per 100 g)
E	5	HTr	10.2 $\pm$ 0.5	HAsTr	42.3 $\pm$ 1.2	None
F	5	HTr	9.0 $\pm$ 1.1	HAsTr	11.7 $\pm$ 1.3	CH α_1 AGP (2.7 mg per 100 g)
G	4	H α_1 AGP	8.6 $\pm$ 1.5	HAs α_1 AGP	98.1 $\pm$ 0.8	None
H	5	H α_1 AGP	7.4 $\pm$ 2.4	HAs α_1 AGP	79.5 $\pm$ 5.5	CH α_1 AGP (2.3 mg per 100 g)

H, human; CH, chicken; R, rat; Tr, transferrin; As, selectively desialylated with neuraminidase.

* Given intravenously 1 min before the dose.

Complete desialylation of human α_1 AGP was achieved by incubating purified neuraminidase⁸ (0.03 units) with 10 mg α_1 AGP at pH 5.2 for 18 h at 37 °C. The enzyme was removed by passing the incubate through a column (10 cm $\times$ 1 cm) of Amberlite IRC-50 equilibrated with 0.015 M Na phosphate, pH 6.0. Under these conditions asialo-orosomucoid passed through the column and neuraminidase was adsorbed by the resin.

The preparation of human asialotransferrin, free from neuraminidase activity, has been described elsewhere¹⁶.

whereas more than 90% of the chicken α_1 AGP was eliminated in the first 4 h of the experiment (Fig. 2).

To determine the mechanism responsible for the premature elimination of the avian α_1 AGP by the rabbit, male Sprague-Dawley rats, of 297 ± 38 g mean body weight, were given an intravenous dose of an iodine-labelled test glycoprotein (10–20 μ g per 100 g body weight) together with a differently labelled protein marker for trapped plasma volume, such as human transferrin (Behringwerke, Germany) or human α_1 AGP (prepared by the same technique as the chicken α_1 AGP). After an interval of 5–6 min, 2.8 ± 0.6 ml blood were withdrawn per 100 g body weight from the heart, under sodium pentobarbital anaesthesia. The liver was removed and immediately homogenised in 50–70 ml 0.89% NaCl containing 16% 2-octanol by volume. Duplicate aliquots of the homogenates were assayed for radioactivity in a Packard model 5986 multichannel analyser.

Experiments A₁, A₂ and A₃ (Table 1) demonstrate the respective behaviour of three chicken α_1 AGP preparations in

the rat, each of which was obtained from the pooled sera of more than 125 chickens. A reproducibly large fraction of the dose was recovered with the liver after administering any of these preparations. Injection of an excess amount of rat α_1 AGP beforehand had no effect on the recovery of this fraction (experiment B). Similar quantities of human α_1 AGP, however, significantly ($P < 0.001$) reduced the accumulation of chicken α_1 AGP in the rat liver (experiment C), and desialylated human α_1 AGP abolished it altogether (experiment D). These observations strongly suggest that, when placed in a mammalian system, chicken α_1 AGP competes for the same hepatic plasma membrane receptor, identified¹² as being responsible for the rapid elimination of mammalian asialoglycoproteins. This view

Fig. 1 Protein-bound radioactivities in the plasma of a hen (1.8 kg) which received a mixture of chicken 125 I- α_1 AGP (●) and human 131 I- α_1 AGP (▲) intravenously.

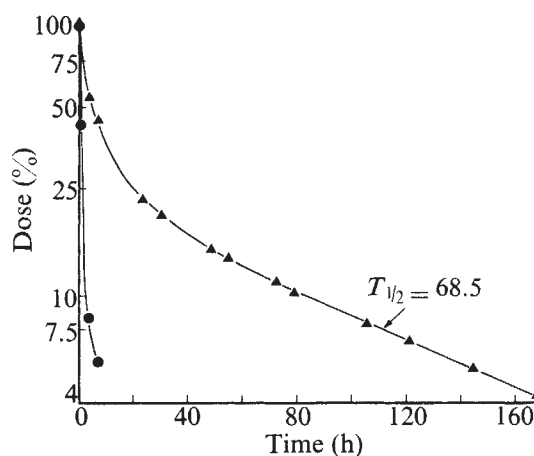
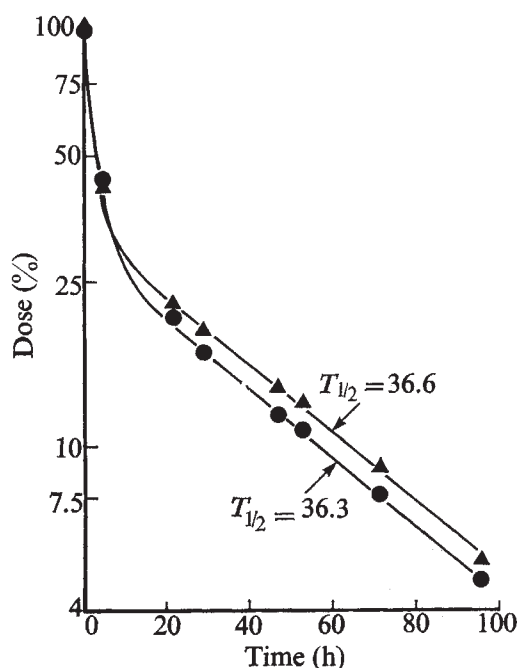


Fig. 2 Protein-bound radioactivities in the plasma of a rabbit (3.8 kg) following the intravenous injection of chicken 125 I- α_1 AGP (●) and human 131 I- α_1 AGP (▲).

is further strengthened by the marked interference with the hepatic clearance of human asialotransferrin and human asialo- α_1 AGP by preinjected chicken α_1 AGP. Thus, hepatic uptake of human asialotransferrin¹³ was almost completely blocked by chicken α_1 AGP (experiments E and F), whereas that of human asialo-orosomucoid was significantly ($P < 0.001$) reduced (experiments G and H).

Since the hepatic asialoglycoprotein receptor operates by recognising exposed galactose residues following the removal of sialic acid from glycoproteins¹⁴, we determined whether native chicken α_1 AGP contained such residues in a terminal position. The protein was treated with galactose oxidase and subsequently reduced by tritiated sodium borohydride¹⁵. By reference to the

radioactivity incorporated into a human asialotransferrin standard in these conditions, the quantity of terminal galactosyl groups in chicken α_1 AGP was estimated to be 2.61 residues per molecule. The corresponding value for human α_1 AGP was 0.97 residues which probably explains the partial blocking effect of this protein (experiment C).

The chemical and *in vivo* evidence presented suggests that chicken α_1 AGP represents a naturally occurring glycoprotein which cannot survive in the mammalian circulation because a significant number of its carbohydrate chains terminate in galactose. Its affinity for the hepatic asialoglycoprotein receptor of rats is stronger than that of human asialotransferrin but weaker than that of human asialo- α_1 AGP. The occurrence of such a glycoprotein in the avian circulation indicates that the chicken liver is either devoid of an asialoglycoprotein receptor altogether or that its requirement for a minimal number of exposed galactosyl groups is fundamentally different from the mammalian system¹⁴.

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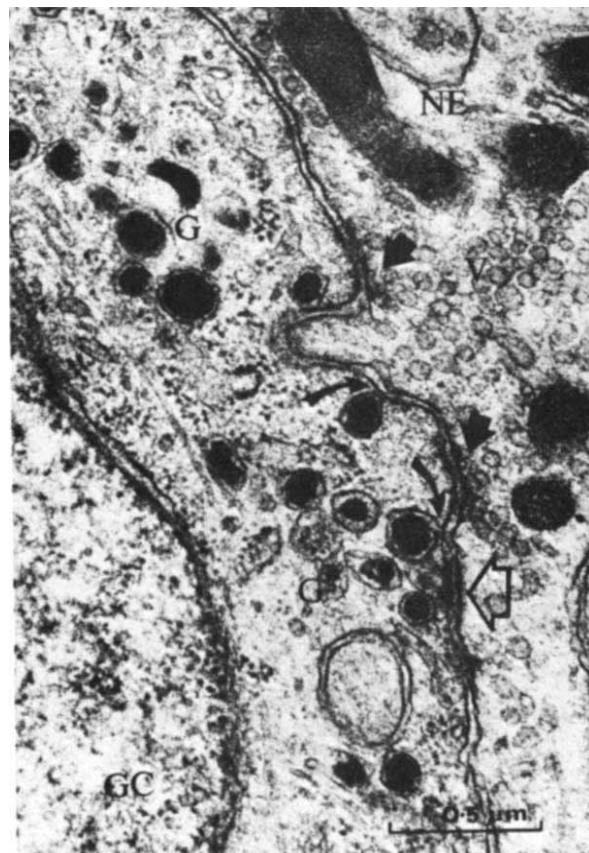


Fig. 1 Electron micrograph of the synaptic contact between a glomus cell (GC) and a nerve ending (NE) from the carotid body of the duck, *Anas platyrhynchos*. The glomus cell contains large numbers of electron-dense granules (G) which are thought to contain dopamine. In contrast, the nerve ending contains agranular vesicles (V) of the cholinergic type. Areas of synaptic contact between the glomus cell and nerve ending are interpreted as being efferent (solid arrows) and afferent (open arrow) on the basis of the location of the storage vesicles and associated membrane thickenings. Some electron-dense granules in the glomus cell seem to contact the cell membrane (curved arrows). Material prefixed with glutaraldehyde, postfixed with osmium tetroxide and embedded in Epon.

New theory for receptor mechanism of carotid body chemoreceptors

EVER since De Castro¹ first ascribed a chemosensory function to the carotid body (later confirmed by Heymans, *et al.*²), there has been much discussion and controversy over the precise mechanism whereby stimulation of the afferent fibres is achieved^{3,4}. The controversy initially centred around the nature of the neurotransmitter released by the glomus cell, which was assumed to be highly sensitive to hypoxia and less sensitive to hypercapnia and acidemia. Perhaps the most generally accepted theory was that the glomus cells respond to hypoxia by releasing a neurotransmitter that initiates an increase in firing rate of the nerve fibres terminating on the glomus cells. The afferent nature of these fibres was originally deduced by De Castro⁵ from light microscopic examination of the nerve fibres innervating the carotid body. The early ultrastructural studies, however, failed to show clear evidence of afferent types of nerve ending in synaptic contact with the glomus cells. On the contrary, these nerve endings were purported to be pre-synaptic and therefore efferent in function because they contain

large numbers of agranular 'synaptic' vesicles⁶⁻⁸. This conclusion was further substantiated by Biscoe *et al.*⁹, who showed that intracranial decentralisation of the IXth cranial nerve caused degeneration of nerve endings on glomus cells. These findings led to an alternative theory of chemoreceptor action⁸. In this case the chemosensory function is attributed to numerous free nerve endings of small diameter, which are found in the carotid body tissue. The glomus cells and associated nerves are held to form part of an efferent inhibitory feedback system, in which release of biogenic amines, that are known to be present in large quantities in glomus cells, depresses chemoreceptor activity.

Work, however, on cats¹⁰ and on ducks (P. J. Butler and M. P. Osborne, unpublished) has shown, in agreement with De Castro⁵, that decentralisation of the nerves leading to the carotid body does not result in degeneration of any of the nerve terminals in synaptic contact with the glomus cells. Furthermore, recent electron microscope studies have shown that both afferent and efferent synaptic complexes occur between the glomus cells and nerve endings¹¹. Our own investigations on the duck have shown that both afferent and efferent synaptic contacts occur between the glomus cell and the same nerve ending (Figs 1 and 2). This latter observation thus supports the suggestion made by Hess and Zapata¹⁰ that "the sensory terminals on the glomus cells might well have in addition to their afferent functions, efferent functional effects like the presynaptic dendrites of the brain, which also pre-

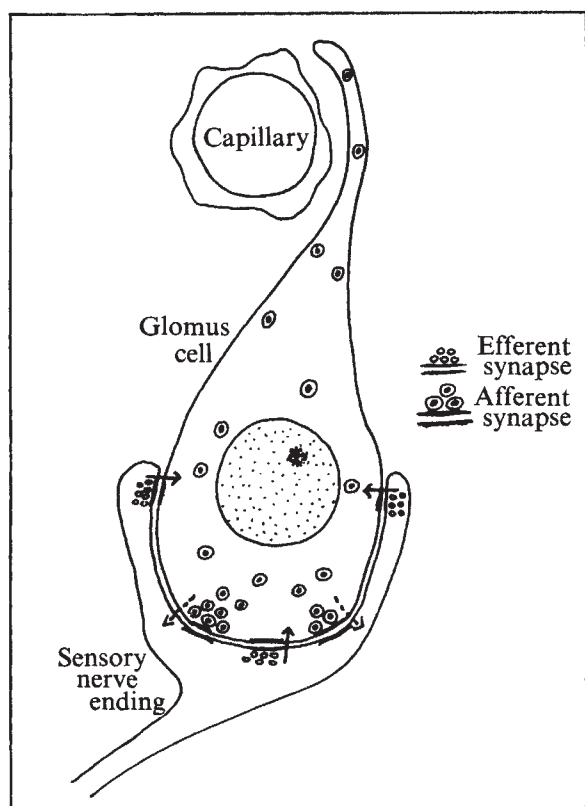


Fig. 2 Structure of a glomus cell and its associated nerve ending. The glomus cell has one or more elongated processes which come into close proximity with the capillaries. The nerve endings are usually cup-shaped (chalice-like) engulfing the basal region of the glomus cell. Afferent and efferent synaptic contacts are shown. The arrows show synaptic conduction during hypoxic conditions, that is, a reduction in neurotransmitter secretion at the afferent synapses (---→) and an increase in neurotransmitter release at the efferent synapses (—→).

sumably play a dual role". Present ultrastructural evidence therefore is strongly indicative of the glomus cell being part of an afferent system.

The assumption that the glomus cells are indeed the chemoreceptive cells is difficult to reconcile with neuropharmacological studies. Eyzaguirre and Zapata¹² have shown that acetyl-

choline (ACh) increases the rate of chemosensory afferent discharge. They therefore suggested that the glomus cells release ACh in response to natural stimuli, and that it is this chemical that excites the afferent fibres. There are unfortunately several strong arguments against this hypothesis, the most important of which are: the glomus cells contain large amounts of catecholamines, in particular dopamine¹³, and this substance, as well as other catecholamines, has a marked depressant effect on spontaneous chemoreceptor discharge¹⁴; cholinergic blocking agents, although they abolish ACh sensitivity, do not abolish the response to natural stimuli^{12,15}. Consequently if a neurotransmitter is released from the glomus cells there would seem to be little support at present for the proposal that ACh is the substance in question⁴.

Taking all of the above evidence into account we have developed a new theory for carotid body chemoreceptive activity. The theory (Fig. 3) is that, in the absence of any physiological effect of the glomus cells, the sensory nerve fibres spontaneously discharge, that is, they are endogenously active¹⁶. This spontaneous activity could result from a constant sodium current flowing at the nerve terminals which could act like a generator potential and depolarise the spike-generating zone to initiate action potentials¹⁷. The frequency of discharge is controlled by secretion of an inhibitory transmitter, dopamine, from the glomus cells. Thus a high rate of dopamine secretion would hyperpolarise the nerve endings, possibly by increasing their potassium conductance¹⁸, and reduce the discharge frequency. When blood gas tensions are normal the rate of dopamine secretion is high, thus attenuating the sensory fibre discharge frequency. In hypoxic conditions, the release of dopamine from the glomus cells is reduced, allowing the nerve endings to return to their depolarised state. Apart from increasing the afferent discharge frequency, depolarisation of the nerve endings in our scheme also causes release of a neurotransmitter, possibly ACh, from the efferent synapses of the nerve terminals. This efferent neurotransmitter acts on the glomus cells to reduce further their rate of dopamine secretion. This results in a larger depolarisation of the nerve terminals which instigates an even greater increase in discharge frequency. Thus in our model the efferent synaptic contacts act as a positive feedback loop. If ACh is the efferent neurotransmitter, then this could explain why cholinergic blocking agents suppress the afferent discharge, but do not abolish the response to hypoxia¹². Also the positive feedback loop could account for the hyperbolic response curve of single-unit preparations to hypoxia¹⁹. We also believe that this model

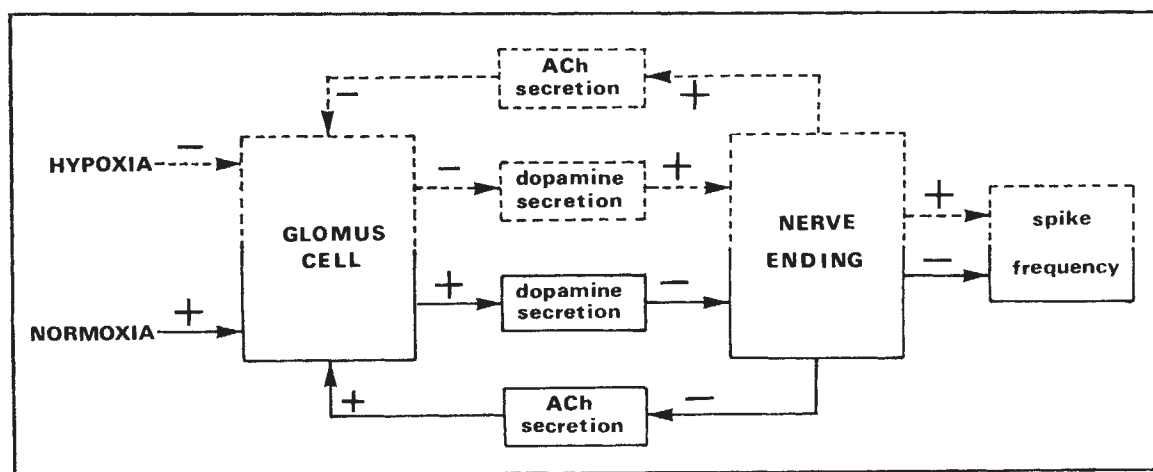


Fig. 3 Schematic flow diagram of a carotid body receptor during hypoxia (upper, dotted lines) and normoxia (lower, solid lines). During normoxia the glomus cell secretes dopamine which reduces afferent discharge frequency. In these conditions little or no ACh is released from the nerve endings at the efferent synapses. During hypoxia the glomus cell reduces its rate of dopamine secretion which leads to an increase in afferent discharge frequency. At the same time ACh is released from the efferent synapses and further suppresses dopamine release from the glomus cell, thus forming a positive feedback loop.

adequately explains the presence of ACh within the carotid body¹² and the presence of acetylcholinesterase²⁰ and dopamine¹³ within the glomus cells. It also accounts for the evidence that ACh¹² and dibenzyline¹⁴ (an α adrenoceptor blocking agent) elevate afferent chemosensory activity and that catecholamines, notably dopamine, block the afferent discharge¹⁴.

The novel feature of our theory is the proposal that in normoxic conditions, the glomus cells secrete an inhibitory transmitter, and in hypoxic conditions the rate of secretion of this transmitter is reduced. Also, during hypoxia we envisage the nerve endings increasing the rate of release of their neurotransmitter substance. These two opposite changes in metabolic rate need not be mutually exclusive if we suppose the low affinity cytochrome a_3 present in the carotid body²¹ is located in the sensory (glomus) cells and that the normal cytochrome a_3 (that is, high affinity) is present in the nerves. The rate of dopamine secretion from the glomus cells could be linked to a metabolic process(es) which is dependent on the oxygen tension. Thus a change in rate of this process might act directly on the secretory mechanism or produce a change in membrane potential of the glomus cell. Therefore it is possible that a depolarisation of the glomus cell would increase, and a hyperpolarisation decrease, the rate of secretion of dopamine. Several attempts have been made to record changes in membrane potential of the glomus cells which are associated with natural or artificial stimuli. In one study²² the authors found no changes, but they could not be sure which type of cell they were recording from. Other authors²³ reported that glomus cells were difficult to depolarise and that the sole potential change they recorded was a hyperpolarisation, and this was only in response to high levels of cyanide ions. The response of the glomus cells, however, need not necessarily involve large changes in membrane potential. Data from vertebrate photoreceptors²⁴ and lateral line organs²⁵ show that receptor potentials are of the order of microvolts rather than millivolts. Since, in these sense organs, changes of a few microvolts are sufficient to alter the rate of neurotransmitter release, it seems that changes in membrane potential of similar magnitude could equally be effective in altering the rate of dopamine release from the glomus cells. Similarly, the efferent neurotransmitter could reduce dopamine secretion from the glomus cells by hyperpolarising effects of less than one millivolt.

Our theory does not exclude the possibility of an independent efferent nerve supply to the carotid body for which there is substantial neurophysiological²⁶⁻²⁹ and some ultrastructural evidence³⁰. Whether this supply is to the vasculature³¹, to the glomus cells³², or to both is unresolved, but whatever the case, the presence of an efferent system need not invalidate our proposals.

Bursts of antidromic impulses in the chemosensory fibres may be expected to increase afferent discharge in our model by the release of ACh. This would occur if it is assumed that the antidromic spikes invade the nerve terminals to cause release of the efferent transmitter. This assumption need not necessarily be correct. It could equally be argued that antidromic spikes would "induce a build-up of positive afterpotentials in the region of orthodromic spike initiation, and produce an elevation of the threshold and depression of discharge"³³. Such a depression of afferent discharge is known to occur following antidromic stimulation in a number of sense organs including the carotid body³³.

Regeneration studies³⁴ indicate that a 12-18-month-old neuroma on the sinus nerve has similar physiological properties to the intact carotid body. Unfortunately the authors do not mention whether they checked the denervated carotid bodies to see if reinnervation had occurred. Until this possibility is eliminated we do not feel that these studies seriously challenge our theory of carotid body function. A more detailed account of our ultrastructural studies will be published elsewhere.

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Pentobarbital modulates transmitter effects on mouse spinal neurones grown in tissue culture

GENERAL anaesthetics depress postsynaptic excitatory transmission in the vertebrate central and peripheral nervous systems¹⁻⁴ although preserving or prolonging both pre-synaptic⁵⁻⁶ and postsynaptic inhibition⁷⁻¹⁰. The mechanisms underlying these cellular events and their precise relationship with the phenomenon of general anaesthesia in mammals have not been elucidated. We have used various invertebrate preparations to show that pentobarbital and other general anaesthetics operate at a postsynaptic level to depress Na⁺-dependent postsynaptic excitation without affecting either Cl⁻ or K⁺-dependent postsynaptic inhibition^{11,12}. Here, using intracellular recording from mouse spinal neurones grown in tissue culture, we show that pentobarbital depresses glutamate excitation and prolongs γ -aminobutyric acid (GABA) inhibition in most cells, through a postsynaptic mechanism.

Spinal cord tissue dissected from 13-d mouse embryos was dissociated and plated at a density of 10⁶ cells per dish (35 mm diameter). The culture medium consisted of modified Eagle's MEM plus animal serum (see ref. 13 for details). Cultures were exposed briefly to a metabolic inhibitor (to suppress growth of background cells) and then allowed to mature for at least 3 weeks before use. For experiments, culture dishes were placed in a chamber on the stage of an inverted, phase-contrast microscope. Temperature and pH of the medium were controlled. Standard electrophysiological techniques were used for intracellular recording and iontophoretic drug application. A bridge circuit permitted current to be injected through the recording pipette so that input resistance could be measured. MgSO₄ was added at the time of study to make a final con-

centration of 10 mM to eliminate spontaneous synaptic activity and suppress presynaptic transmitter release. Neuronal responses to transmitters were not affected.

The somata of large multipolar spinal cord cells were impaled under direct vision using electrodes filled with 4 M K⁺ acetate. Two or more independent iontophoretic pipettes filled with various drugs (0.5 M glutamate, pH 8; 1 M GABA, pH 3.5; 0.5 M glycine, pH 3.5; 0.5 M Na⁺ pentobarbital, pH 9.2) were then positioned close to one another at the surface of the cell. Drugs were iontophoresed, using the appropriate polarity current, to cell somata so that the recording pipette could detect maximally the effects of the drugs. The results reported here were obtained from a population of neurones with resting potentials more negative than -45 mV and action potentials of 50 mV or more in amplitude. Dorsal root ganglion cells were also present in the cultures but the results regarding these cells are still inconclusive.

Glutamate, a putative transmitter mediating synaptic excitation¹⁴, depolarised more than 95% of spinal cells examined (Fig. 1). The dose-dependent, rapid depolarisation typically elicited spike discharge, was associated with a small decrease in membrane resistance and had an extrapolated inversion potential which was usually around -20 mV (ref. 15 and N. Brookes, unpublished).

Application of GABA or glycine, putative inhibitory transmitters in the central nervous system (CNS)¹⁴, markedly reduced membrane resistance and depressed spike discharge in a dose-dependent, reversible manner in 85% and 50%, respectively, of cells examined (Fig. 1b). The equilibrium potential for these responses was typically near the resting potential and in all instances where the effects of these two drugs could be compared on the same cell, they had identical equilibrium potentials¹⁵. Pentobarbital also decreased the membrane resistance (Fig. 1c) in a dose-dependent, reversible manner in about 80% of cells tested. The decrease in resistance associated with pentobarbital amounted to 5–15% of the resting input resistance, although decreases of 25% were observed with very large applications. The resistance change was often accompanied by a 1–3 mV shift in membrane potential, usually in the hyperpolarising direction. The slowly developing pentobarbital effect required more iontophoretic current than the rapidly developing GABA or glycine responses (Fig. 1c). Pentobarbital did not seem to affect the height of the spike, its duration or threshold.

Focal applications of pentobarbital depressed the depolarising responses to glutamate (Fig. 1a) in a dose-dependent, reversible manner (12 of 14 cells tested) without diminishing the membrane responses to either GABA (Fig. 1b) or glycine. The depressant effects of pentobarbital could be seen in the absence of any measurable effects on membrane properties, when low iontophoretic currents of the drug were used. Direct effects of pentobarbital on membrane properties, when present, could account for only a small percentage of the depression of the glutamate response. In Fig. 1a, for example, a 55% depression of the glutamate response by pentobarbital was associated with a 13% decrease in membrane resistance, while in Fig. 1b (panels on the right) a 12% decrease in membrane resistance was present during the 38% depression. The decrease in membrane resistance induced by GABA or glycine was never antagonised by pentobarbital (Fig. 1b and d). More than 50% of the cells studied showed a prolongation of the GABA response in the presence of pentobarbital (Fig. 1b and d). This effect was analysed quantitatively by examining the change in membrane conductance following iontophoresis of GABA as a function of time. Semi-log plots of these data revealed linear relationships from which half-times ($t_{1/2}$) for the restoration of the control conductance were obtained (our unpublished data). In Fig. 1b, for example, the $t_{1/2}$ increased from 2.5 s for the response under drug-free conditions to 3.9 s for the response in the presence of pentobarbital. Pentobarbital did not prolong the glycine response in four cells tested.

Pentobarbital also tended to change the polarity of the GABA

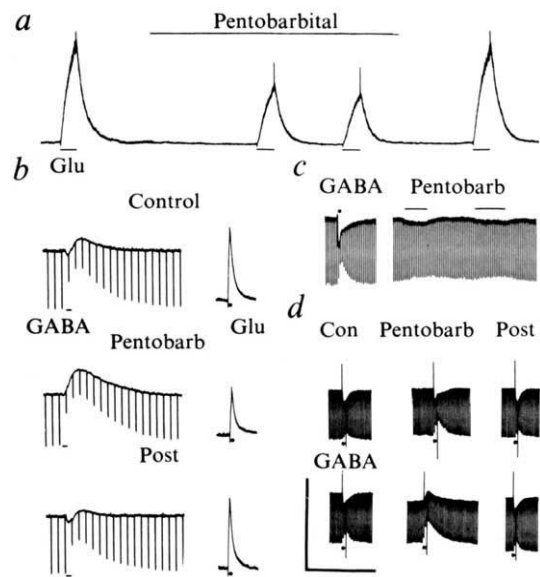


Fig. 1 Effects of iontophoretically applied pentobarbital on nerve cells in tissue culture and its interactions with putative transmitters. *a*, Pipettes containing pentobarbital and glutamate were positioned at the same site on the cell soma. Standard pulses of glutamate (20 nA, 3 s) were applied alone (indicated by labelled bars or filled circles) and in the presence of pentobarbital (50 nA). Membrane potential was -58 mV. Upward deflections are positive for all records. *b*, Similar experiment using glutamate, GABA and pentobarbital pipettes near the cell soma. Records show drug responses to constant pulses of the two transmitters (8 nA, 0.3 s for glutamate and 45 nA, 1.5 s for GABA) before (control), during (pentobarbital) and seconds after (post) application of pentobarbital (30 nA). Downward deflections in GABA traces here and in panels (c) and (d) are voltage responses to constant current pulses reflecting membrane resistance during drug responses. $t_{1/2}$ for the relaxation of the conductance change after the termination of GABA application was 2.5 s in control and 3.9 s during pentobarbital application (see text). Peak resistance decrease during GABA response was 52% in control compared with 54% during pentobarbital. Membrane potential was -50 mV. Calibration: 25 mV, 10 s. *c*, Comparison of effects of GABA and various doses of pentobarbital applied to the same membrane site. Onset of GABA response (45 nA and 2 s) was much faster than pentobarbital effect (45 nA first bar, 25 nA second bar). Membrane potential was -50 mV. Calibration: 25 mV, 50 s. *d*, GABA and pentobarbital applied to same membrane site on cell soma. Top three records show the action of a constant pulse of GABA (20 nA and 3 s) before (con), during (pentobarb), and after (post) the application of pentobarbital (12 nA). Bottom three records illustrate the effects of a 24-nA application of pentobarbital. The membrane hyperpolarised by 3 mV during the larger dose of pentobarbital and the GABA response inverted (see text). $t_{1/2}$ of the relaxation of the GABA conductance change was 1.8 s for the con and 4 s for the pentobarb response. Membrane potential was -56 mV. Calibration: 35 mV, 50 s.

response in a depolarising direction (Fig. 1b and d, bottom panels). This could not be explained by a change in the resting potential induced by pentobarbital and may have resulted from an effect of pentobarbital either on an ionic gradient on which the GABA response depends, or on the basic ionic mechanism induced by GABA. Alternatively, the GABA response in some neurones may be normally biphasic, as suggested by the control record in Fig. 1b. The effect of pentobarbital may thus be to accentuate the later, depolarising component of this response.

The results demonstrate three specific effects of pentobarbital on mammalian CNS neurones: (1) a depression of postsynaptic glutamate excitation independent of direct effects on membrane potential or resistance; (2) a variably present prolongation of the conductance change induced by GABA (an effect not seen in invertebrates); and (3) a dose-dependent, slowly developing increase in membrane conductance (usually 5–15%). These effects of pentobarbital may explain the invariable depression

of CNS excitability during pentobarbital anaesthesia. The results suggest that the depression of excitatory postsynaptic potentials in the CNS could be the result of the selective, postsynaptic effects of the drug without necessitating major effects on presynaptic release. The observed prolongation of the GABA response may help to explain the prolongation of presynaptic and postsynaptic inhibition, both of which are thought to be mediated by GABA^{6,10,14,16-18}. Direct membrane effects of pentobarbital might also contribute to general anaesthesia by causing either a slight shunting of neuronal membranes, thereby reducing the size of excitatory synaptic potentials, or a slight hyperpolarisation, thus moving the membrane from its firing level.

There are several ways, therefore, in which the cellular effects of barbiturates could lead to generalised depression of neuronal excitability and general anaesthesia: (1) selective, postsynaptic depression of glutamate excitation, (2) prolongation of GABA-mediated postsynaptic inhibition, (3) prolongation of GABA-mediated presynaptic inhibition leading to partial deafferentation and (4) direct effects on CNS neurones to depress their excitability. The relative importance of each of these in producing general anaesthesia, as well as the effects of pentobarbital on other aspects of synaptology studied under controlled conditions in tissue culture, remain to be investigated.

Note added in proof: Since this report was submitted we have found similar pharmacological effects of pentobarbital on transmitter responses using anaesthetic concentrations (0.1–0.2 mm) applied from large-bore pipettes.

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Increase in binding capacity for triiodothyronine in tadpole tail nuclei during metamorphosis

REGRESSION of the tadpole tail is under direct control of the thyroid hormones and provides a system for study of their mechanism of action¹. In this system triiodothyronine (T_3) is two to five times as active as thyroxine (T_4) in causing tail regression^{2,3}. Studies of the binding of ^{125}I - T_3 and ^{125}I - T_4 after incubation of hormone with sliced tailfin tissues have revealed high-affinity, saturable binding sites in the cell nucleus⁴—a maximum of 1,500 and 800 sites per nucleus for T_3 and T_4 , respectively. (Similar results have been obtained with tadpole liver cells⁵.) The dissociation constants for T_3 and T_4 were almost identical (10^{-10} M). These results suggest that in

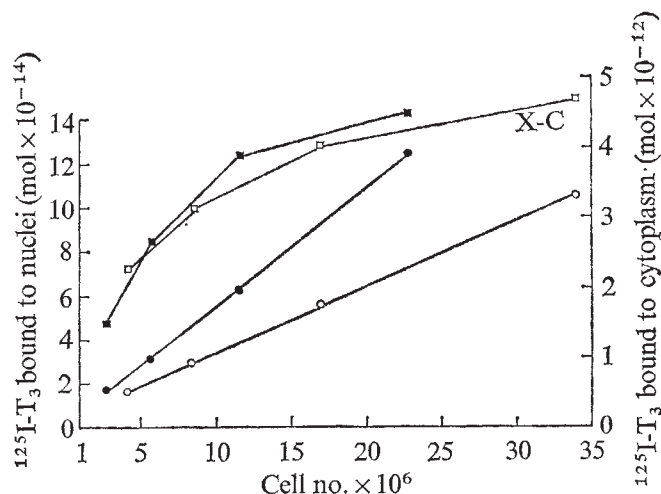


Fig. 1 Dependence of binding of ^{125}I - T_3 to nuclei and to cytoplasm on the cell number. Tailfin cell suspensions were prepared from stage X and XVIII–XIX tadpoles. One ml of the cell suspension containing different amounts of cells was incubated with ^{125}I - T_3 (final concentration, 7.0×10^{-9} M) for 3 h at $25^\circ C$. $\circ$, Binding to stage X nucleus; $\bullet$, stage XVIII–XIX nucleus; $\square$, stage X cytoplasm; $\blacksquare$, stage XVIII–XIX cytoplasm. Unfractionated cell populations were used. Contamination by blood cells was less than 3%. These cells appeared to be a mixture of epidermal, mesenchymal, and connective tissue cells¹⁶. Separation of tailfin tissue cells from stage X and XX–XXI was performed on a Ficoll gradient (5–15%, linear). Both stages had three visible band of cells and the relative widths of each band were almost identical for these two stages. Therefore, it seems that there was no significant change in cell type of the tailfin in these preparations.

amphibians the maximum number of binding sites rather than the affinity constant correlate with the biological activity of T_3 and T_4 . In mammals, however, specific high-affinity binding sites for the thyroid hormones were found in nuclei^{6–8} and correlate with the affinity constant.

Derby⁹ found that the sensitivity of tailfins to T_4 increases as metamorphosis progresses, but did not show whether this resulted from a change in the maximum binding capacity of the nucleus or to a change in the affinity constant for T_3 . We have found that the maximum nuclear binding capacity for T_3 doubles during metamorphosis and that the affinity of the nucleus for the hormone decreases slightly during spontaneous metamorphosis.

Rana catesbeiana tadpoles were classified according to stages as defined by Taylor and Kollros¹⁰. Dorsal and ventral tailfins were sliced and digested twice in 5–10 volumes of amphibian Ringer phosphate buffer (ARB), pH 7.6, containing 0.10% glucose, 0.1% methylcellulose, 0.20% collagenase and 0.10% hyaluronidase (Worthington) for 30 min. After filtration through nylon cloth (mesh size, 125 μm) the cell pellet was washed five times with ARB. Cell yield from the sliced tissues was 50%, estimated from DNA recovery. Cells were incubated with ^{125}I - T_3 (69 $\mu Ci \mu g^{-1}$, Abbott) as described later.

Table 1 Changes in maximum binding capacity and dissociation constant for T_3 by tailfin nuclei during metamorphosis

Tadpole stage	Binding sites per cell nucleus ($\pm$ s.e.m.)	Apparent dissociation constant (pM) ($\pm$ s.e.m.)
X	1,330 $\pm$ 30	112 $\pm$ 5
XV	1,580 $\pm$ 40	105 $\pm$ 4
XVIII–XIX	2,830 $\pm$ 70	155 $\pm$ 7
XX–XXI	2,410 $\pm$ 30	169 $\pm$ 4

Values were obtained from Fig. 3, where the intercept of the ordinate represents the maximum number of binding sites and the slope represents the negative of the dissociation constant. The data for stage XV were obtained by the same method as for the other stages. 9.6×10^6 cells were incubated for 3 h at different concentrations of ^{125}I - T_3 . The correlation coefficient for the linear regression was 0.97 ($P < 0.01$). While the correlation is high, it is not certain that the data fit only a single class of binding sites.

Preparation of tailfin cell nuclei was performed by the method of Samuels and Tsai⁶ with 90% recovery of cell DNA¹¹. ^{125}I - T_3 analysed on a Sephadex G-25 column^{4,5} contained 99.5% ^{125}I - T_3 and 0.5% ^{125}I -iodide. The radioactivity of the nuclear pellet dissolved in 1 N NaOH and the extranuclear fraction was measured with a Beckman Model LS-250 liquid scintillation spectrometer with a counting efficiency of 48% using 0.5% Omnifluor, 1% Cab-o-Sil, and 8% naphthalene in dioxane.

The time course of binding of ^{125}I - T_3 to nuclei and cytoplasm was studied at 25 °C and 4 °C. Both nuclear and extranuclear binding progressed linearly for 2 h and then plateaued. Incubation was for 3 h in all subsequent experiments. The extent of hormone binding was depressed 50% at 4 °C.

The relationship between T_3 binding and the total number of cells used was studied at 7.0×10^{-9} M ^{125}I - T_3 , using stage X and XVIII–XIX cells (Fig. 1). A linear relationship was observed only in nuclear binding over the range tested. From the intercept of the line, it was estimated that a cell nucleus from stage X contains 1,900 binding sites for T_3 and that of stage XVIII–XIX contains 3,300 sites, giving values slightly in excess of the values obtained by Scatchard plots (Table 1). At 7.0×10^{-9} M, the specific binding sites were saturated and contained some T_3 , bound nonspecifically.

Binding of ^{125}I - T_3 to nuclei and the extranuclear fraction was examined at different T_3 concentrations (Fig. 2) using cells prepared from stages X, XV, XVIII–XIX, and XX–XXI. The ratio of bound T_3 to the T_3 concentration of the medium is plotted against the concentration of T_3 in the medium at equilibrium. The ratios for the extranuclear fraction remained constant and almost identical for different stages. The ratios for the nucleus decreased as the hormone concentration increased, demonstrating that the nucleus contains high-affinity and saturable binding sites. These data were replotted in a standard Scatchard plot (Fig. 3)¹². Table 1 lists the maximum number of binding sites in the nucleus and their apparent dissociation constants as estimated from Fig. 3. Maximum binding sites per nucleus remained almost unchanged through stage XV. The present estimation of the maximum number of binding sites and their dissociation constants at stage X agrees with the results of binding experiments using tail slices⁴. At stage XVIII–XIX, binding sites almost doubled compared with stage X and then decreased slightly at stage XX–XXI. The affinity constant remained almost unchanged until stage XV and decreased

Fig. 2 Binding of ^{125}I - T_3 to tail nuclei and cytoplasm at different concentrations of T_3 . Tailfin cells were prepared from stage X, XVIII–XIX and XX–XXI tadpoles and were incubated for 3 h with different concentrations of ^{125}I - T_3 . The number of cells used were 1.15×10^7 (stage X), 1.03×10^7 (XVIII–XIX) and 9.90×10^6 (XX–XXI). The incubation medium was saved for the determination of T_3 counts. The ratio of bound ^{125}I - T_3 to the hormone concentration in the medium (M) was plotted against the medium hormone concentration at equilibrium. Solid lines show the binding to nuclei. Dotted lines show the binding to cytoplasm. ○, Stage X; □, stage XVIII–XIX; △, stage XX–XXI.

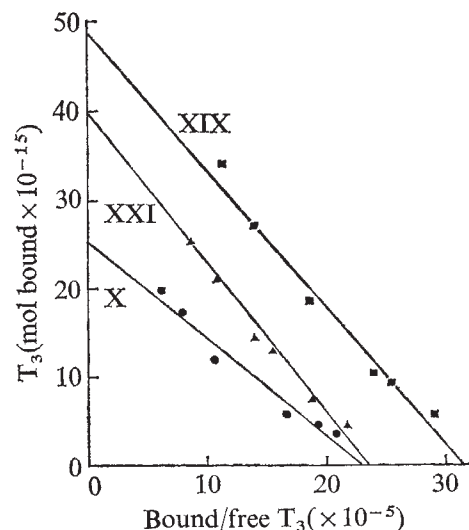
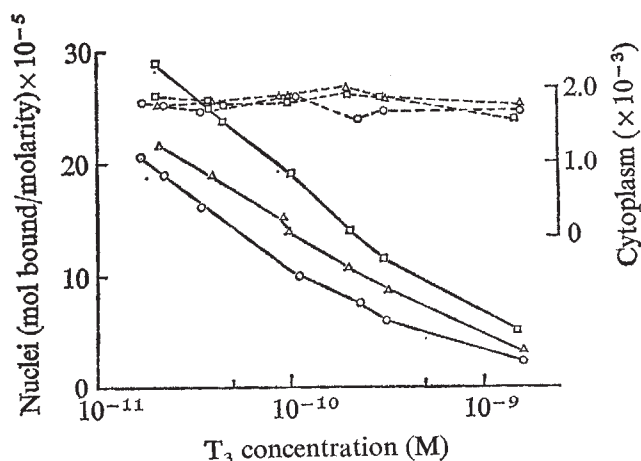


Fig. 3 A Scatchard plot of the binding of ^{125}I - T_3 by tail nuclei. Binding of ^{125}I - T_3 to the nuclei was determined by incubating cell suspensions with radioactive hormone in 1.0 ml of ARB, containing 0.1% glucose and 0.1% methylcellulose. After incubation, the cell pellet was obtained by centrifugation and the medium was washed to determine T_3 counts. The cell pellet was washed by two successive suspensions and centrifugation in 2 ml of the cold incubation medium. A nuclear pellet was prepared as described above. Lines were obtained by linear regression with a correlation coefficient of 0.98–0.99 ($P < 0.01$). Stage X, ●; stage XVIII–XIX, ■, stage XX–XXI, ▲.

thereafter. Since the available evidence suggests that the thyroid hormone level increases just before climax¹³, it is probable that a higher fraction of the T_3 binding sites in the nucleus were occupied by endogenous T_3 at stages XVIII–XIX and XX–XXI than in early premetamorphic stages (X and XV).

The results presented here suggest that the maximum number of binding sites for T_3 in tail nuclei increased during spontaneous metamorphosis. The increase in hormone sensitivity of tail tissue during metamorphosis may be the result of an increase in nuclear binding capacity for T_3 rather than an increase in the affinity constant, which seems to decrease as tadpoles metamorphose. Tata reported that the acquisition of early metamorphic competence in whole *Xenopus* larvae is accompanied by the appearance of temperature-sensitive binding sites for thyroid hormones¹⁴. The binding of very high concentrations of T_4 to temperature sensitive sites in tadpole liver nuclei was reported by Griswold *et al.*¹⁵. Samuels and Tsai⁶ suggested that nuclear receptors for T_3 are not constant, but increase after exposure of intact cells to non-radioactive T_3 . Therefore it is possible that the higher level of endogenous thyroid hormone at the onset of climax stages in the tadpole increases the nuclear receptors of the hormone, resulting in a greater tissue sensitivity to the hormone.

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Glucagon-like immunoreactivity in insect corpus cardiacum

SINCE Steele attributed hyperglycaemic activity to the corpus cardiacum of the cockroach neurosecretory system¹⁻³, it has been demonstrated in other species of Orthoptera⁴⁻¹⁰ and in Diptera^{11,12} and Hymenoptera¹³. Although extracts of corpora cardiaca from the blowflies *Phormia*¹¹ and *Calliphora*¹⁴ and the silk moth *Cecropia*¹⁵ seem to be inactive, the hyperglycaemic hormone is liberated from the *Calliphora* gland by electrical¹² or mechanical¹⁴ stimulation *in situ*. Similarly, extracts from *Locusta*^{6,16} and *Phormia*¹¹ are poorly active intraspecifically, but are hyperglycaemic when injected into the cockroach. The nutritional state of the recipient is apparently crucial in determining the extent of the response¹¹.

In the best studied insects, the hyperglycaemic hormone functions similarly to glucagon in vertebrates: the hormone increases carbohydrate levels in the circulating haemolymph

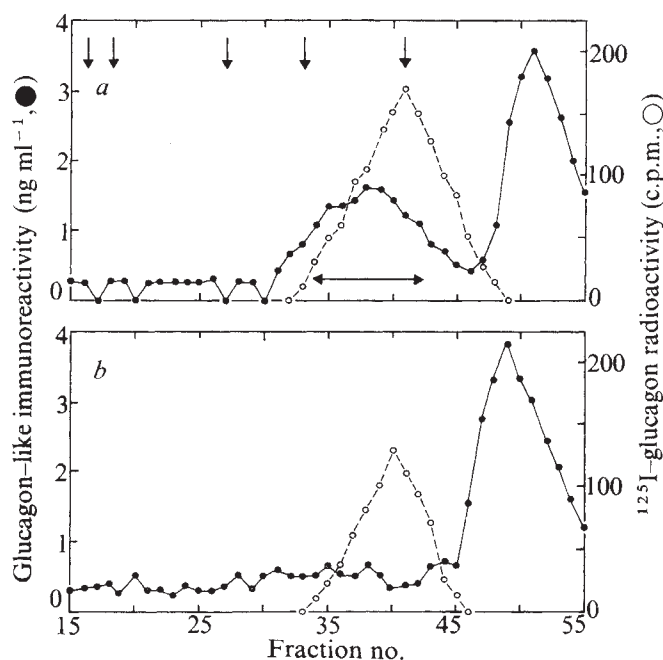


Fig. 1 Gel filtration of extracts of corpus cardiacum/corpus allatum complexes and brains from adult *M. sexta*. Tissue (21 complexes or brains) was suspended in 0.1 M Tris, 0.05 M NaCl, 0.25% bovine serum albumin, 1 mM diisopropylphosphorofluoridate (DFP), pH 7.6, lyophilised and extracted with 0.3 ml of 7 M guanidine hydrochloride containing a trace amount of ¹²⁵I-glucagon. The extracts were separately applied to a column (0.9 × 42 cm) of BioGel P-10 equilibrated at 4 °C with Tris-albumin buffer containing pancreatic kallikrein inhibitor (300 U ml⁻¹) instead of DFP, and the components eluted at a flow rate of 5 ml h⁻¹. The 0.6 ml fractions were counted for radioactivity and 0.15 ml aliquots of each were removed for radioimmunoassay essentially as described²⁵. The antiglucagon serum was a gift from Dr L. Heding, Novo Laboratories, Copenhagen. Glucagon-like immunoreactivity (left axis, solid lines) and ¹²⁵I-glucagon radioactivity (right axis, dashed lines) were recorded for the complexes (a) and the brains (b). The elution positions of protein standards from the same column are indicated by the vertical arrows (a) which represent from left to right, albumin (void volume), soybean trypsin inhibitor (STI), cytochrome c, pancreatic trypsin inhibitor, and glucagon or ¹²⁵I-glucagon, respectively. Fractions 34 to 42 indicated by the horizontal arrow (a) were pooled for further analysis.

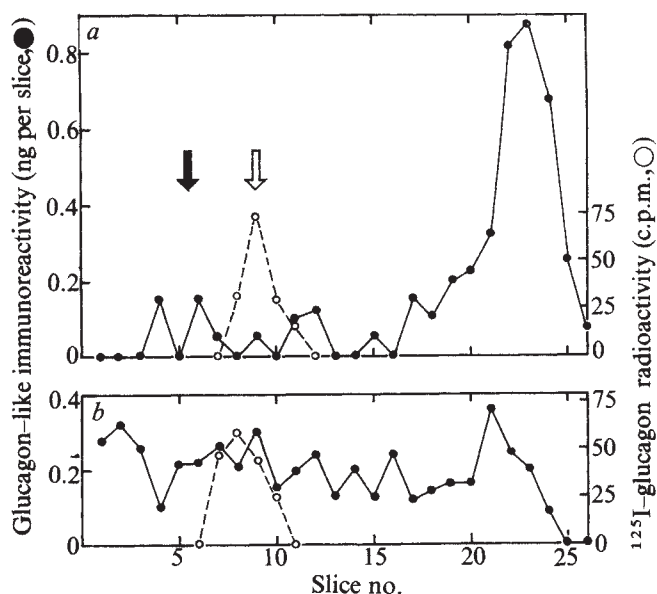


Fig. 2 Polyacrylamide gel electrophoresis at pH 8.7 of extracts of corpus cardiacum and corpus allatum from *M. sexta*. Tissue (9 gland pairs) was extracted with 0.05 ml of 8 M urea containing trace amounts of ¹²⁵I-glucagon and bromphenol blue. The extracts were applied to 10% polyacrylamide gels²⁸ containing 8 M urea (0.4 × 6 cm) and were subjected to electrophoresis at 22 °C. Gels were sliced into 1.5-mm sections and placed in 0.5 ml of the buffer used for chromatography before counting for radioactivity. After elution of the peptide components during 24 h at 4 °C, the supernatant fluid was withdrawn and subjected to immunoassay. Glucagon-like immunoreactivity (left axis, solid lines) and ¹²⁵I-glucagon radioactivity (right axis, dashed lines) were recorded for the corpus cardiacum (a) and the corpus allatum (b) extracts. Migration was from cathode (left) to anode. The position of the tracking dye is coincident with the right axis. The solid and open arrows (a) indicate the migration position of porcine glucagon and ¹²⁵I-glucagon respectively. The additional negative charge in iodinated glucagon at pH 8.7 increases its anodal mobility relative to that of the unlabelled hormone. Control experiments showed that the presence of urea or other gel components did not alter the sensitivity of the immunoassay.

in vivo and stimulates glycogenolysis and the activation of phosphorylase in the fat body *in vitro*^{5,6,17,18}. By several criteria, the insect hormone also seems to be a low molecular weight polypeptide^{2,6,8,10,13,16}. We report here the identification of a highly acidic peptide with glucagon-like immunoreactivity from corpora cardiaca of the adult tobacco hornworm *Manduca sexta*.

A survey for immunoreactive glucagon in tissues of *M. sexta* showed that reactive peptides were present in corpus cardiacum/corpus allatum complexes and haemolymph, but not in brain, aorta, recurrent nerve or fat body. The component from the complexes was eluted slightly ahead of ¹²⁵I-glucagon (molecular weight 3,500) during gel filtration on BioGel P-10 (Fig. 1a). The elution volume of the immunoreactive component corresponded to that of a 4,500-dalton peptide. As Fig. 1b shows, gel filtration of an extract of brains from *M. sexta* yielded no similar peak of glucagon-like immunoreactivity. The peak of apparent reactivity at the far right of each profile in Fig. 1 is caused by interference in the assay by guanidine hydrochloride and represents the inclusion volume of the column.

A closer examination of the distribution of the immunoreactive peptide showed that it was primarily associated with the corpus cardiacum rather than with the corpus allatum (Fig. 2a, slices 21-25). Its rate of migration during electrophoresis at pH 8.7 is in agreement with its low molecular weight as determined by gel filtration, and further indicates a high acidity. Nevertheless, the abilities of the serially diluted, pooled fractions 34-42 (Fig. 1a) and porcine glucagon to compete with ¹²⁵I-glucagon for binding to the antibody

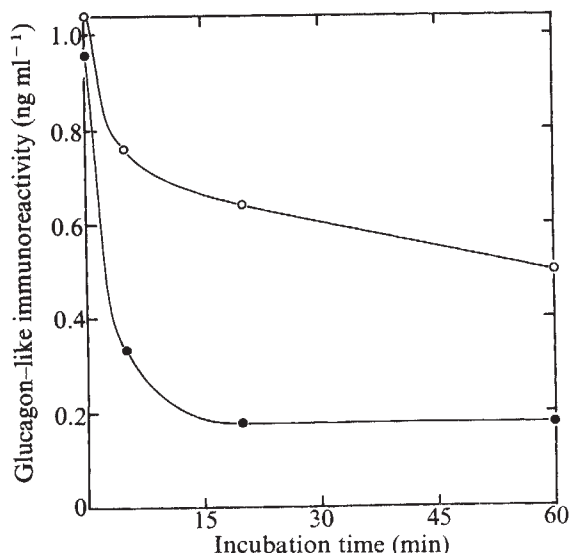
decreased in parallel. Although the immunoreactivity profile of Fig. 2b suggests that corpus cardiacum contains a single, major glucagon-like peptide, two or more rapidly migrating forms might not be separated under the conditions used here.

Since mammalian glucagon¹⁹ and the insect hyperglycaemic hormone^{8,13} are sensitive to proteolytic degradation, we compared the susceptibilities of the immunoreactive component and porcine glucagon to trypsin (Fig. 3). Although the immunoreactivities of both the insect peptide and glucagon are diminished by digestion with trypsin, the former is degraded either more slowly or to a lesser extent. This result may be a consequence of the higher acidity of the insect peptide (Fig. 2a). Quantitation of glucagon-like immunoreactivity after gel filtration or after polyacrylamide gel electrophoresis indicated that each pair of corpora cardiaca from *M. sexta* contains 0.4–0.5 ng equivalent of glucagon. This value should be regarded as a lower limit, however, since affinity of our antibody for the insect material may well be less than for porcine glucagon.

Examination of sequence data shows that the structure of the vertebrate hyperglycaemic hormone glucagon has been well conserved during evolution: the human,²⁰ bovine,²¹ and porcine¹⁹ hormones are identical, although the structures of the corresponding, slowly evolving cytochromes *c* differ by an average of 7% (ref. 22). Similarly, avian glucagon differs from the human hormone by 7% (ref. 23), whereas the difference between the corresponding cytochromes is about 12% (ref. 22). Assuming from these limited data that the rate of mutational acceptance for glucagon is only about half that for cytochrome *c* or 1.5 accepted point mutations per 100 residues per 100 Myr,²⁴ we expect that a glucagon-like peptide might have changed by only 15% during the approximately 900 Myr since the divergence of insects and mammals²⁴. Neither this estimated extent of change nor the apparently higher molecular weight of the insect peptide would be likely to preclude reactivity with antibodies directed toward mammalian glucagon²⁵.

Weins and Gilbert first noted the nearly identical physiological

Fig. 3 Effect of trypsin on the glucagon-like immunoreactivity in a gel-filtered extract of corpus cardiacum/corpus allatum complexes from *M. sexta*. Samples (0.4 ml) of the pooled fractions 34–42 from Fig. 1 or of a solution of porcine glucagon (1 ng ml⁻¹ in chromatography buffer) were incubated with diphenylcarbamyl chloride-treated trypsin (0.075 mg) at 25 °C for the indicated intervals. The reaction was stopped by the addition of 0.1 ml of a solution of STI (8 mg ml⁻¹). All samples were subjected to the immunoassay and glucagon-like immunoreactivity was recorded for *M. sexta* (○) and glucagon (●). The data points at zero time were obtained by adding STI to the appropriate solution before the addition of trypsin. Control experiments showed that the presence of trypsin plus STI did not alter the sensitivity of the assay.



actions of the insect hyperglycaemic hormone and vertebrate glucagon⁵. The tissue distribution of the glucagon-like immunoreactivity in *M. sexta* parallels the distribution of the hyperglycaemic hormone in other insects^{1,18,26,27}. Furthermore, both immunoreactivity and hyperglycaemic activity are sensitive to tryptic degradation and behave similarly during gel filtration at neutral pH¹³. The high acidity of the immunoreactive peptide is also consistent with the finding that hormone activity does not adsorb to cation-exchange resins^{10,13}. Although these chemical and physical similarities suggest that the insect hyperglycaemic hormone and the glucagon-like peptide are the same, final proof of their identity must await purification and sequence determination of the hyperglycaemic peptide.

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Ethanol inhibition of transport of 5-hydroxyindoleacetic acid from cerebrospinal fluid

MUCH work has been directed at demonstrating the possible interaction of ethanol with levels of neurotransmitter amines or their metabolism in brain^{1,2}. We have previously demonstrated an increase in brain levels of 5-hydroxyindoleacetic acid (5-HIAA) in mice after both a single and chronic administration of ethanol³. Our more recent work⁴ has focused attention on the inhibition of transport of 5-HIAA from the central nervous system (CNS) by ethanol as the factor responsible for the increase in brain 5-HIAA after ethanol. 5-HIAA diffuses from the CNS into cerebrospinal fluid (CSF), from which it is re-

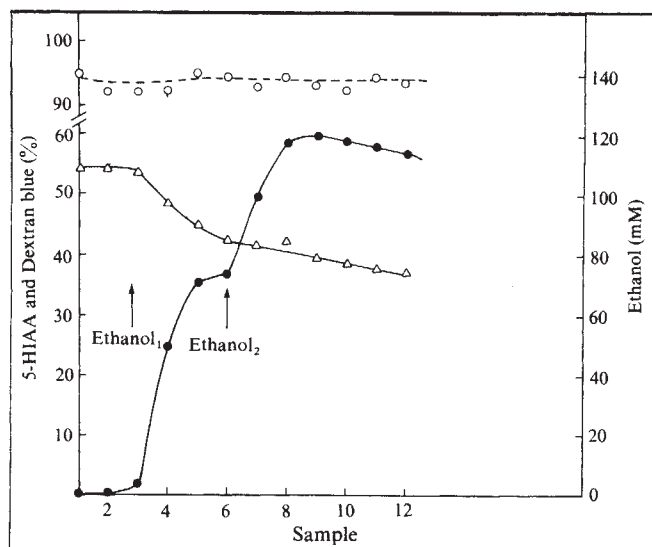


Fig. 1 Transport of 5-HIAA from spinal subarachnoid space after ethanol administration. The results illustrate one typical experiment of those treated statistically in Fig. 2. A steady-state concentration of 5-HIAA and Dextran blue in outflow fluid during perfusion was established as follows. Adult cats were anaesthetized with pentobarbital sodium (30 mg kg^{-1} intraperitoneally) and prepared for perfusion as described previously⁶. After the head of the animal was fixed in a stereotaxic instrument, an inflow cannula connected to a constant infusion pump was positioned in the cisterna magna. Polyethylene tubing fixed in the lumbar subarachnoid space⁶ served as outflow; its end was positioned 10 cm below the level of the cisterna magna and at the horizontal level of the lumbosacral cord. Rectal temperature was recorded during the experiment and kept within normal range ($\pm 0.5^\circ \text{C}$). Artificial CSF (ref. 7) containing 5-hydroxyindoleacetic-carboxyl- ^{14}C -acid (5-HIAA- ^{14}C , 400 ng ml^{-1} , specific activity $1 \mu\text{Ci } 19.2 \mu\text{g}^{-1}$) and Dextran blue ($100 \mu\text{g ml}^{-1}$) pH 7.4, was used for perfusion. The rate of perfusion was $0.097 \text{ ml min}^{-1}$. With this perfusion rate approximately 120 min was required to get constant concentration of 5-HIAA- ^{14}C in the outflow fluid. Thereafter samples of perfusate were collected at 20 min intervals. To shorten the equilibration period the perfusion was started in a few experiments with a rate of $0.191 \text{ ml min}^{-1}$ and continued for 30 min. After this initial period, the perfusion rate was changed to $0.076 \text{ ml min}^{-1}$ and collection of samples was begun 60 min from the start of perfusion. Ethanol concentrations in the perfusate were determined by gas chromatography⁴. Radioactivity was determined by adding 0.1 ml of perfusate to 15 ml of Bray's solution⁸ and subjecting the sample to scintillation counting. Changes in concentration of Dextran blue were determined by measuring changes in absorbance at 630 nm from the initial value of 0.093 ± 0.002 which corresponded to $100 \mu\text{g ml}^{-1}$ of Dextran blue. Percentage transport for 5-HIAA (Δ) was calculated from

$$\left(1 - \frac{^{14}\text{C in outflow}}{^{14}\text{C in inflow}}\right) \times 100$$

Concentration of Dextran blue ($\circ$) in collection samples was expressed as percentage of the concentration being infused. Ethanol (1.5 g kg^{-1}) was injected intraperitoneally twice with approximately 60 min between injections, and the concentrations of ethanol appearing in the perfusate ($\bullet$) were determined.

moved by a carrier-mediated transport into the bloodstream⁵. The effect of ethanol on transport of 5-HIAA from CSF was studied by perfusing this acid through the spinal subarachnoid space from the cisterna magna to the lumbar region of cats. This was shown to be a sensitive and reliable model for the study of transport of 5-HIAA from CSF (ref. 6).

Figure 1 shows levels of 5-HIAA and Dextran blue in perfusate before and after two intraperitoneal injections of ethanol (1.5 g kg^{-1}). The concentration of Dextran blue did not change significantly during the experiment indicating that the bulk flow of the fluid was constant during the experiment. On the other hand, a significant decrease in 5-HIAA transport was observed after ethanol administration ($P < 0.001$; analysis of variance for repeated measures⁹).

Figure 2 illustrates the mean decrease in 5-HIAA transport

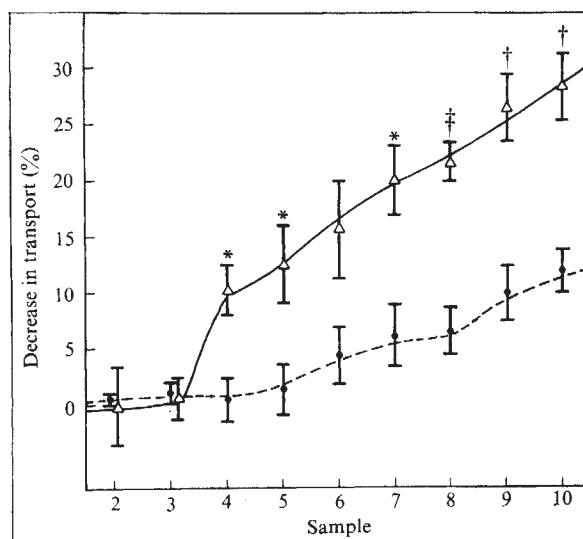
after ethanol administration. The administration of ethanol produced a 25–30% decrease in the transport of 5-HIAA during the course of our experiments. Decreases in transport of 5-HIAA correlated well with the administration of ethanol (Fig. 2) and the appearance of ethanol in the collection samples (Fig. 1). The peak ethanol levels in CSF of cats after injection of ethanol (Fig. 1) were similar to those previously noted in CNS of mice receiving 3 g kg^{-1} ethanol⁴. In some preliminary experiments (data not shown), ethanol (5 mg ml^{-1} artificial CSF) was added to the perfusion fluid. Such treatment produced no significant change in the transport of 5-HIAA compared with control cats. Concentrations of ethanol in the perfusion outflow fluid, however, were found to be extremely low (that is, $< 1 \text{ mM}$), indicating that ethanol was quickly lost from the subarachnoid space. Therefore, concentrations of ethanol necessary to inhibit 5-HIAA transport could not be maintained by this method.

Our present experiments with cats injected with ethanol clearly indicate that ethanol inhibits transport of 5-HIAA from the CSF, and extend our earlier observations on the effects of ethanol on the transport of 5-HIAA from the CNS (ref. 4). Previous work has demonstrated that measurable increases in the levels of 5-HIAA in brain do not occur simultaneously with peak ethanol levels in brain but approximately 60 min thereafter³. Thus, it is interesting to note that in these experiments the inhibition of 5-HIAA transport by ethanol increases with time. In addition, the partial inhibition of active transport systems, such as those for organic acids, which may be working at near maximal capacity^{10,11}, would result in a progressive accumulation of 5-HIAA with time. Our studies with mice have also demonstrated that both the accumulation of 5-HIAA and the inhibition of transport systems for this organic acid by ethanol are reversible processes.

Zarcone *et al.*¹² demonstrated an increase in 5-HIAA in the lumbar CSF of alcoholics consuming ethanol. A similar increase in 5-HIAA in cat cisternal CSF after injection of ethanol has also been observed (G. E. Goodman, M. P. Schulman, and M. Radulovacki, personal communication). Our findings that ethanol inhibits transport of 5-HIAA out of CSF (Figs 1

Fig. 2 Inhibition of 5-HIAA transport from spinal subarachnoid space by ethanol. Perfusions of the spinal subarachnoid space were performed as described in the text. Values represent mean $\pm$ s. e. of percentage change from steady-state transport of 5-HIAA established initially. Steady-state concentration of 5-HIAA was $50.9 \pm 3.6\%$ of infused 5-HIAA in control cats ($\bullet$, $n = 3$) and $55.4 \pm 2.4\%$ in those cats which were to receive ethanol (Δ , $n = 3$). Time of the injection of ethanol as in Fig. 1. Results were compared using Student's t test.

* $P < 0.1$. † $P < 0.05$. ‡ $P < 0.02$.



and 2) and CNS (refs 3 and 4) explain the underlying mechanism of these observations. It is also interesting to note that inhibition of transport systems for certain organic acids not only inhibits the exit of acids from the CNS and CSF but also facilitates the penetration of such acids from blood into the CSF and CNS (ref. 5). The increase in 5-HIAA or similar organic acids (biogenic acids) in the CNS may interfere with the normal metabolism of biogenic amines. Biogenic acids have been shown to inhibit brain aldehyde reductases^{13,14} which are responsible for the production of the alcohol derivatives of the biogenic amines (for example, MHPG). Biogenic acids, such as phenylacetic acid, have also been shown to inhibit certain decarboxylases^{15,16} necessary for the biosynthesis of neurotransmitters such as catecholamines and γ -aminobutyric acid. Further studies are in progress to elucidate the relationship between increases in CNS concentrations of organic acids and 'hangover' and withdrawal symptoms produced by ethanol.

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Placental production and foetal utilisation of lactate and pyruvate

APPROXIMATELY 75% of foetal oxygen consumption of sheep can be accounted for by the uptake of glucose from the umbilical circulation¹⁻³ and catabolism of amino acids⁴. The contribution to foetal energy metabolism of exogenous long-chain fatty acids⁵ and fructose¹ seems to be negligible. We report now that the placenta normally provides lactate for the foetus, in sufficient

quantities to account for 25% of foetal oxidative metabolism. This finding invalidates the assumption that under normal physiological conditions there is a net flux of lactate from foetus to mother. The assumption seemed reasonable in the past because the concentration of lactate in foetal blood is greater than in maternal blood and because of the formerly prominent hypothesis that anaerobic metabolism is very active in foetal life.

Twelve Dorset and Western ewes at days 67-147 of gestation were starved for 48 h and then placed under pentobarbital sedation (5 mg kg⁻¹) and spinal anaesthesia (6 mg pontocaine in hyperbaric glucose). Seven of these animals received polyvinyl catheters in the maternal femoral artery, the uterine vein, the foetal pedal artery and umbilical vein as described previously^{2,6}. In four cases the foetal vessels only were catheterised. In one sheep, only the uterine circulation was studied.

All lambs except two were live born spontaneously or by Caesarean section. The two lambs found dead after spontaneous delivery were appropriate in size for their gestation ages of 95 and 115 d and had not been sampled for 25 and 7 d respectively before labour.

After surgery all animals were placed in rectangular stalls and allowed food and water *ad libitum*. Foetal and maternal catheters were flushed daily with heparin. Procaine penicillin (600,000 U) and streptomycin (0.5 g) were administered on the day of surgery and the next three days. Study began at least 24 h after surgery and extended to day 17 after surgery.

Foetal (0.25 ml) and maternal (0.45 ml) blood samples were drawn simultaneously into 3-ml plastic syringes (Sherwood) surrounded by ice. These were prepared on the day before sampling by inverting the syringe with needle attached into its prepackaged container. The space between syringe and container was filled with water and stored at -4 °C to form an ice jacket around the syringe. The blood samples were transferred immediately to preweighed plastic tubes (12 mm x 75 mm) containing 0.5 ml of 5.1% perchloric acid and agitated on a Vortex mixer (Scientific Industries) until precipitation was complete. These tubes were then placed in a refrigerated centrifuge (International Equipment Co.) and spun at 3,000 r.p.m. for 20 min. After separation of the supernatant, centrifugation was repeated before analysis. Blood (0.2 ml) for analysis of oxygen content was drawn into heparinised glass capillary tubes (Scientific Products) in which 0.15 mg of sodium fluoride had been dried.

Olsen's enzymatic method⁷, utilising L-lactate dehydrogenase, was used to determine the concentrations of lactate and pyruvate. Oxygen contents were determined with a Lex-O₂-Con (Lexington Instrument Corp.) calibrated with distilled water saturated with oxygen at 0 °C. All samples were analysed in triplicate.

Lactate/oxygen quotients across the umbilical circulation were calculated on the basis of umbilical venous arterial differences ($v-a$) of lactate and oxygen, as follows:

$$\text{lactate/oxygen quotient} = 3 (v-a \text{ lactate}/v-a \text{ oxygen})$$

Expressed in this fashion, the lactate/oxygen quotient represents the fraction of foetal oxygen consumption required to metabolise

Table 1 Whole-blood lactate, pyruvate and oxygen contents (mM $\pm$ s.e.m.)

	Lactate			Pyruvate			Oxygen		
	No. of animals	Samples	mM	No. of animals	Samples	mM	No. of animals	Samples	mM
Umbilical Artery (a)	11	40	2.05 $\pm$ 0.15	6	23	0.25 $\pm$ 0.08	6	23	3.31 $\pm$ 0.17
Vein (v)			2.21 $\pm$ 0.15			0.25 $\pm$ 0.08			4.95 $\pm$ 0.15
Uterine Artery (A)	8	42	0.86 $\pm$ 0.07	6	24	0.13 $\pm$ 0.02			1.64*
Vein (V)			0.93 $\pm$ 0.07			0.14 $\pm$ 0.01			
$v-a$			0.16*			0.00			
$V-A$			0.07*			0.01*			

* $P < 0.001$ (paired t test).

Table 2 Lactate/oxygen quotients (L/O_2) in simultaneously drawn samples of umbilical venous (v) and arterial (a) blood

Animal	Age	v Lactate mM	v-a Lactate mM	v O_2 mM	v-a O_2 mM	L/O_2
019	96	1.78	0.40	5.98	1.52	0.79
	97	1.37	0.15	5.54	1.56	0.29
	98	1.52	-0.01	5.27	1.70	-0.02
	101	1.17	0.07	5.31	1.83	0.11
	103	2.29	0.15	5.22	2.55	0.17
	104	5.65	0.26	5.13	3.13	0.25
	108	3.69	0.08	3.05	1.83	0.13
032	114	1.49	0.09	5.45	1.92	0.14
031	125	2.15	0.11	3.71	1.56	0.22
	130	2.63	0.21	4.24	2.05	0.31
034	127	1.84	0.05	6.09	1.50	0.12
	128	1.86	0.09	4.96	1.13	0.24
	130	2.12	0.20	4.60	1.23	0.49
	131	1.82	0.04	4.78	1.38	0.09
	133	2.35	0.17	4.71	1.72	0.30
091	143	2.96	0.18	4.91	1.72	0.31
084	147	1.89	0.10	4.95	1.22	0.26
Mean		2.26	0.13	4.93	1.73	0.25*
±s.e.m.		0.25	0.02	0.18	0.11	

*95% confidence limits according to Fieller's¹³ theorem 0.16 to 0.32.

the lactate acquired by the umbilical circulation to carbon dioxide and water.

Table 1 shows that v-a lactate in the uterine and umbilical circulations was highly significant ($P < 0.001$). There was no significant veno-arterial difference of pyruvate concentration in the umbilical circulation. There was, however, a relatively small, but statistically significant difference of pyruvate concentration between uterine veins and maternal arterial blood. The lactate/oxygen quotient across the umbilical circulation was calculated on 17 samples from 6 animals (Table 2). The results indicate that lactate was being metabolised by the foetus in amounts that could account for 25% of the foetal oxygen uptake.

Many studies performed *in vitro* have demonstrated that the placenta of several species has a relatively fast rate of aerobic glycolysis. Murphey and Hawkins⁸ demonstrated the phenomenon in the rat placenta, and Bell *et al.*⁹ found that it occurred only in the presence of chorionic epithelium. Aerobic production of lactate by human placental tissue has been measured *in vitro*¹⁰⁻¹².

Our data demonstrate that lactate concentrations are greater in the umbilical and uterine veins than in their corresponding arteries. In conjunction with the *in vitro* studies, these data indicate that the lactate acquired by the foetus from the umbilical circulation is produced by the placenta.

This is the first demonstration that lactate produced by the placenta is an important substrate of foetal metabolism. As demonstrated by the measurement of lactate/oxygen quotient, catabolism of the lactate acquired from the umbilical circulation to carbon dioxide and water would require one-quarter of the total foetal oxygen uptake. This is equal to the estimated amount of oxygen needed for the foetal breakdown of amino acids⁴ and half the oxygen required for the aerobic metabolism of the glucose which is delivered normally by the placenta to the sheep foetus¹⁻³.

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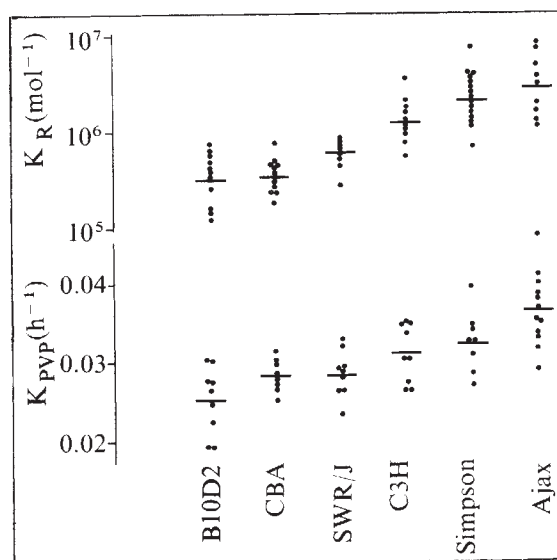
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Relationship between macrophage clearance of PVP and affinity of anti-protein antibody response in inbred mouse strains

MACROPHAGES are important in immunity on their own, in delayed-type hypersensitivity, in phagocytosis of immune complexes, and in the afferent limb of antibody responses. Evidence for the latter includes the increased immunogenicity of macrophage-processed antigen¹, and the requirement for macrophages in antibody responses involving cooperation between T and B lymphocytes². In addition, antibody titres can be increased or decreased by agents, such as adjuvants³ or colloidal carbon⁴, which probably act on macrophages. Changes in either quantity or quality (affinity) of antibody, or both, may occur: carbon⁵ reduces the affinity of mouse anti-protein antibody but does not affect quantity, whereas adjuvant⁶ increases both. Differences in macrophage activity could therefore be responsible for differences in antibody affinity between mouse strains⁷. Carbon clearance differs between strains^{5,8}, and has been found to be loosely related to affinity⁵. We have used a new test of macrophages which shows differences of clearance function which relate closely to differences of antibody affinity.

Polyvinyl pyrrolidone (PVP) clearance⁹ was measured in normal 10 to 14-week-old mice of six inbred strains (B10D2(new), SWR/J, CBA, Simpson and Ajax). Results are expressed as the rate constant (K_{PVP} , h^{-1}) of the exponential fall in blood radioactivity between 18 and 48 h after intravenous injection of ¹²⁵I-PVP (Radiochemical Centre, Amersham). Mice of four of these strains were immunised with two weekly injections of 1 µg PVP (molecular weight, 360,000), and clearance of ¹²⁵I-PVP was tested one week later. Affinity (K_R , mol^{-1}) and quantity (Ab , µM antibody binding sites) of antibody against human

Fig. 1 PVP clearance (K_{PVP}) in mice of six inbred strains, compared with affinity (K_R) of antibody for HSA. (Data for K_R kindly provided by Dr M. W. Steward⁷.)



serum albumin (HSA) was determined in normal Ajax mice after saline immunisation¹⁰, and in mice given 10 mg PVP (molecular weight, 360,000) intraperitoneally 24 h before each HSA injection.

K_{PVP} differs significantly between the six strains of mice (Fig. 1, $F=10.4$, $P < 0.01$, one-way analysis of variance), possibly on a genetic basis. Variation within each strain exceeds the methodological variation⁹, suggesting that environmental factors also contribute. The correlation between the strain ranking order for K_{PVP} and antibody affinity (K_R) is striking ($r_s=0.99$, $P < 0.01$, rank correlation on mean values), but there is no significant correlation between K_{PVP} and Ab_i ($r_s=0.30$, $P > 0.05$) for the five strains for which values of Ab_i are available⁷.

Tests of macrophage function have included *in vivo* clearance measurements using a variety of particles and *in vitro* tests of isolated monocytes or peritoneal cells¹¹. The different clearance tests may measure, to some extent, different things⁸, and their relationship to *in vitro* function has been little studied. All other clearance measurements give much faster clearance rates than ours, and use doses large enough to saturate the phagocytic capacity of the Kupffer cells¹². Such doses are likely both to stimulate and to block the function measured, and the magnitude of such effects differs between strains⁸. The amount of PVP used is much smaller and does not cause dose-related blockade⁹, so that PVP clearance may parallel the handling by macrophages of small quantities of antigen more accurately than do other tests. Evidence that it does indeed measure a macrophage function includes the facts that the PVP is detected in macrophages and clearance and the hepatic uptake is reduced by carbon and by corticosteroids, but increased by oestrogens. There is no evidence of antibody response at the time of testing, and repeat tests usually give similar values. Large doses of unlabelled PVP blockade clearance of both PVP and carbon.

The close relationship between PVP clearance and affinity of antibody to HSA suggests that the former measures a biologically important function, and supports the theory that variation

in macrophage function underlies differences in affinity of antibody response, as does the alteration of both antibody affinity and macrophage activity with carbon, adjuvant or oestrogen^{5,6}. Cells responsible for phagocytosis of PVP are involved in determining antibody affinity, since Ajax mice given 10 mg PVP before each injection of HSA showed significant ($P < 0.025$, Mann-Whitney U test) reduction in K_R (log mean $0.12 \times 10^6 \text{ mol}^{-1}$, compared with control mean of $0.95 \times 10^6 \text{ mol}^{-1}$), but Ab_i was unchanged (mean $2.0 \text{ } \mu\text{M}$, control mean $1.8 \text{ } \mu\text{M}$, $P > 0.05$).

Poor macrophage function probably leads to a low affinity antibody response because of poor selection of B lymphocytes. The optimum response after adjuvanted immunisation⁶ makes it unlikely that the limiting factor is a deficiency of suitable lymphocytes. Perhaps PVP clearance correlates better with antibody affinity than does carbon clearance because PVP is more similar in size to the immunogenic microaggregates of soluble proteins than are carbon particles, and because the large dose of carbon partially blockades the macrophages¹². Our results do not imply that endocytosis of antigen is necessary for optimal antibody response¹³. PVP clearance and antigen handling may both depend on overall macrophage activity and motility, or macrophage phagocytosis of PVP may depend on cell surface characteristics which are also important in cell interactions. We do not know how the differences of antibody response we describe are related to those of mice selected for high and low titres of agglutinating antibody¹⁴. These differences may also depend on macrophage function, as peritoneal cells from animals with low response show increased rates of antigen degradation¹⁵. If they are related, the ability of the macrophage surface to bind and retain intact antigen molecules may be the critical function which determines the nature of the antibody response.

Immune PVP clearance also differs between strains. K_{PVP} was increased after immunisation (Fig. 2) in all four strains tested, but more markedly so in the strains with high PVP clearance (Simpson and Ajax) than in the other two. Immunised B10D2(new) and CBA mice clear PVP less well than many unimmunised Ajax mice. There may be both low affinity antibody and defective clearance of immune complexes, and possibly also less antibody, but we do not know if there are differences in affinity of antibodies to PVP (T-cell independent and mainly IgM, ref. 11) similar to those of IgG antibodies to cooperation-dependent protein antigens. This individual variation of function may contribute to susceptibility to immunopathological processes such as immune complex disease⁶, including that in man, in whom similar variation occurs (A.G.M. and J.F.S., unpublished).

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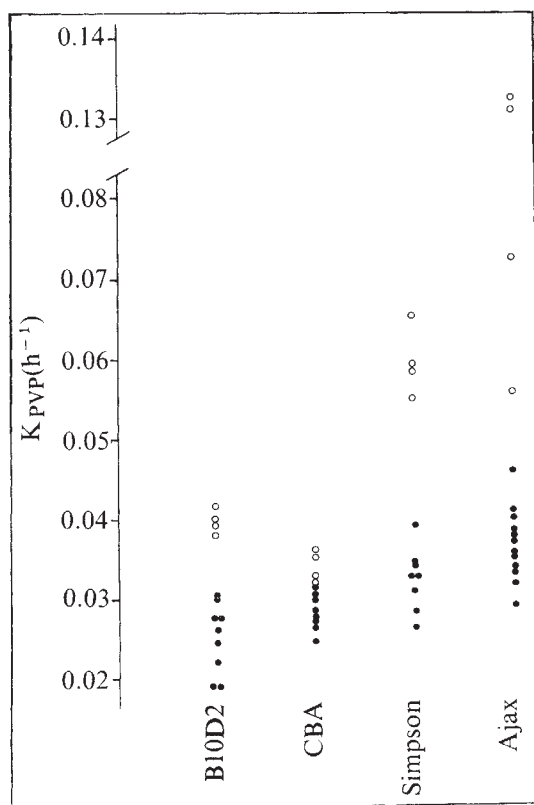
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Fig. 2 PVP clearance (K_{PVP}) in normal mice (●) and mice immunised with $1 \mu\text{g}$ PVP (○).



Tumour-associated transplantation antigens of chemically induced sarcomata cross reacting with allogeneic histocompatibility antigens

INDIVIDUAL tumour-associated transplantation antigens (TATA) of chemically induced tumours and histocompatibility antigens (HA) share the ability of inducing transplantation immunity, and have other properties in common, such as the high degree of polymorphism¹⁻³ and a similar cell surface behaviour⁴. A reciprocal relationship between the quantitative expression of TATA and H-2 antigens has been observed⁵⁻⁶, suggesting the existence of common mechanisms either in their synthesis or in the control of their phenotypic expression. Such indirect similarity prompted us to study the existence of cross reactions between TATA and HA. We therefore determined whether 3-methylcholanthrene (MCA)-induced sarcomata possess TATA-containing allogeneic histocompatibility determinants. Here we report data from two BALB/c fibrosarcomas, ST-5 and B-2.

We investigated whether protection against the syngeneic tumours could be induced by immunisation with normal allogeneic tissues. Groups of BALB/c mice were transplanted with skin or with kidney plus liver from syngeneic, C57BL/6J or C3Hf, mice, with skin of W/Fu rat, or were injected intraperitoneally with sheep red blood cells (SRBC). Ten days later all mice received 400 rad (whole-body) and were challenged on the following day with sarcoma ST-5 cells. As shown in Fig. 1a a significant protection against sarcoma ST-5 (as assessed by χ^2 test) was elicited by C57BL/6J skin or liver plus kidney grafts when compared with syngeneic skin graft ($P < 0.01$ at day 12 and 16, and $P < 0.05$ at day 20 after challenge); C3Hf skin graft was highly effective ($P < 0.001$ from day 8 to 32, and $P < 0.05$ at day 36) and a still stronger protection was achieved by C3Hf liver plus kidney graft ($P < 0.01$ from day 8 to 36), whereas no resistance could be induced by rat skin graft or by injection of SRBC. No effect was induced by C57BL/6J or C3Hf liver plus kidney graft against the challenge of sarcoma B-2 (Fig. 1b).

It seems, therefore, that TATA from sarcoma ST-5 cross reacted with HA of C57BL/6J and C3Hf strains. It is unlikely that a nonspecific increase of the immune response caused by antigenic stimulation was responsible for the anti-tumour resistance induced by allografts, since rat xenograft and SRBC injection had no effect, and since sublethal X-irradiation strongly impairs primary immune response. Moreover, no resistance could be induced with the same schedule against sarcoma B-2, which lacks TATA.

These results were partially confirmed by serological studies.

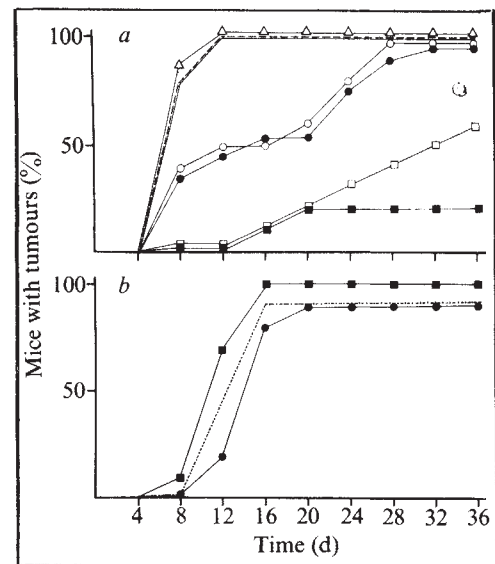


Fig. 1 Antitumour resistance induced by allograft. Groups of 10 BALB/c males 2 months old, received either a full thickness skin graft (15×20 mm), or a subcutaneous injection in the back of liver plus kidney fragments in 0.5 ml MEM+antibiotics, or were injected intraperitoneally with 10^8 SRBC in 0.5 ml saline. Liver plus kidney fragments were prepared by teasing and mincing the organs and the equivalent of one-third of the pool of one liver and two kidneys was given to each mouse. Challenge: 5×10^4 ST-5 cells (a) or 2×10^5 B-2 cells (b) in 0.2 ml MEM, subcutaneously in the flank. All mice were examined for palpable tumours every 4 d after challenge. Skin graft: $\square$, C3Hf; $\circ$, C57BL/6J; — , BALB/c; --- , W/Fu rat. Liver plus kidney graft: $\blacksquare$, C3Hf; $\bullet$, C57BL/6J; --- , BALB/c; Δ , SRBC. Sarcoma ST-5 originated from BALB/c fibroblasts exposed to MCA in a diffusion chamber placed intraperitoneally in a BALB/c mouse. The treated fibroblasts, for experimental reasons not relevant to this study⁷, were transferred to a (C3Hf $\times$ BALB/c) F_1 hybrid until they developed in a palpable nodule. The BALB/c genotype of the tumour was assessed by retransplanting the nodule in BALB/c mice where it took promptly. Sarcoma B-2 was obtained by syngeneic subcutaneous transplantation of *in vitro* spontaneously transformed BALB/c fibroblasts. Both tumours were serially transplanted in unconditioned BALB/c mice, and used between the 8th and 15th *in vivo* passage. At the time of the present experiments ST-5 was found to be immunogenic using the *in vivo* growth-excision assay⁷, whereas B-2 showed no immunogenicity; no cross reactivity was found between the two sarcomas. The individuality of the ST-5 TATA was also proved by the absence of cross reaction with other MCA-induced BALB/c sarcomas.

Absorption with ST-5 cells significantly removed the cytotoxic activity of a BALB/c anti-C57BL/6J serum compared with absorption with BALB/c lymphocytes or with sarcoma B-2 ($P < 0.001$ at 15×10^6 absorbing cell dose, Fig. 2). In contrast, several attempts to absorb the cytotoxic activity of a BALB/c anti-C3Hf serum with either tumour failed.

To demonstrate further transplantation antigens belonging to C57BL/6J and C3Hf strains on ST-5 cells, we compared the immunogenic strength of TATA of sarcoma ST-5 in the syngeneic against (BALB/c $\times$ C57BL/6J) F_1 (CB6F₁) and (C3Hf $\times$ BALB/c) F_1 (C3CF₁) hybrid mice. We anticipated that if C57BL/6J or C3Hf determinants were part of TATA of sarcoma ST-5, the immunogenicity of this tumour in the hybrid mice should have been absent or lower than in BALB/c mice. Growth-excision of ST-5 in CB6F₁ mice elicited no immunity against a subsequent challenge of the related cells, whereas only a slight reduction in protection was observed in C3CF₁ compared with BALB/c mice (Table 1).

TATA of sarcoma ST-5 seems to be complex. Whereas anti-tumour resistance induced by C57BL/6J and C3Hf allografts may be explained as TATA possessing a unique determinant shared by both C57BL/6J and C3Hf HA and absent in BALB/c tissues, such as specificity 5 of *k* and *b* H-2 genotypes, or H-4^a antigens, in the hybrid experiment a clear

Table 1 Immunogenicity test of ST-5 sarcoma in syngeneic against hybrid mice

Experimental groups*	No. of mice with tumour†		
	BALB/c	C3CF ₁	CB6F ₁
Immune	5/16 (31)	14/27 (51)	12/26 (46)
Controls	14/20‡ (70)	23/28§ (82)	13/23¶ (56)
Protection achieved by immunisation	39%	31%	10%

*Antitumour immunity was obtained by growing a tumour fragment subcutaneously (controls receiving normal BALB/c tissues subcutaneously). When approximately 10 mm in diameter, tumours were surgically removed and controls sham-operated. Eight days later all the animals were challenged subcutaneously with 10^5 ST-5 cells prepared by trypsinisation.

†Final check for tumour takes at 32 d after challenge. Percentage of takes in brackets. Difference in takes between control and immune mice of the same strain, as evaluated by χ^2 test.

‡ $P < 0.05$.

§ $P < 0.02$.

¶Not significant.

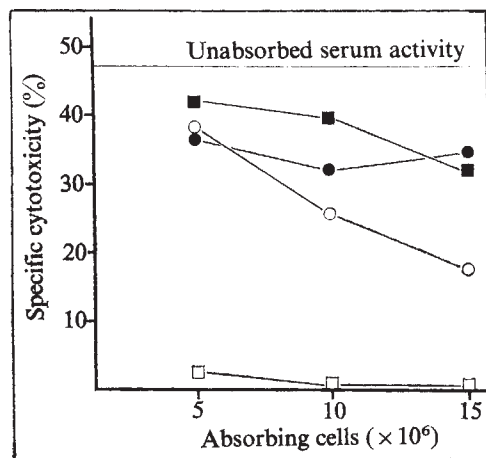


Fig. 2 Absorption of BALB/c anti-C57BL/6J serum by ST-5 and B-2 cells. Serum obtained by immunising BALB/c mice with 4 weekly injections of C57BL/6J lymphoid cells. The animals were bled 7 d after the last injection. The ⁵¹Cr-release test on C57BL/6J radiolabelled lymph node target cells¹⁴ was performed at the serum endpoint dilution of 1:32 in the presence of guinea pig complement (B. D. Merieux, Marseille, France). The specific ⁵¹Cr release was calculated as follows: $100 \times \frac{\text{experimental release-background}}{\text{maximum releasable } ^{51}\text{Cr-background}}$ (where background represents ⁵¹Cr release in the presence of heat inactivated complement). Tumour cells prepared by trypsin treatment for 20 min at room temperature and suspended in MEM, were incubated for 2 h at 37 °C before absorption. Lymph node cells were obtained by mincing and teasing subcutaneous and mesenteric lymph nodes, and re-suspended in MEM. Absorptions were carried out by incubating 0.12 ml serum with 5×10^6 packed cells for 30 min at 37 °C followed by 30 min at 4 °C, in 2 ml test tubes. To avoid excess dilution by a large cell pellet the absorption doses of 10 and 15×10^6 cells was reached by two and three repeated absorptions on 5×10^6 cells, respectively. No gross difference was observed between ST-5 and B-2 cell pellet volume. Each point is mean of three replicates. ○, ST-5 cells; ●, B-2 cells; ■, BALB/c lymph node cells; □, C57BL/6J lymph node cells.

reduction in immunogenicity was observed in CB6F₁ mice only. This suggests that TATA of sarcoma ST-5 contains at least two components, one limited to C57BL/6J strain, tolerated in CB6F₁ and recognised as foreign in C3CF₁ hybrids; the other shared by both C57BL/6J and C3H₁ strains and therefore tolerated in both CB6F₁ and C3CF₁ hybrids. A prevalence of C57BL/6J in the make-up of ST-5 TATA may also explain the failure of ST-5 cells to absorb the activity of a BALB/c anti-C3Hf serum. Note, however, that C3Hf grafts induced a stronger antitumour resistance than the C57BL/6J ones.

The possibility that a somatic hybridisation spontaneously occurred in the early passage of the tumour in the C3CF₁ host, may be responsible for the observed cross reactions, is unlikely, since the phenomenon has been reported to be infrequent⁸, and since the subsequent serial passages in unconditioned BALB/c mice should have selected against hybridised cells. In addition, a hybridisation between BALB/c and C3CF₁ cells could not explain the presence, on ST-5 cells, of C57BL/6J antigens not shared with C3Hf strain.

We consider the origin of TATA of sarcoma ST-5 to be the result of carcinogen-induced random mutations at the level of histocompatibility genes, either of the H-2 system or of the minor loci. Such a possibility is supported by the genetic linkage which seems to occur between TATA and the H-2 system⁹.

Moreover, assuming that HA have a key function in the economy of cell life¹⁰, only TATA identical or very similar to HA of normal cells should be selected for among the different neoantigens which appear at the initial stages of carcinogenesis¹¹. Alternatively, the possibility of a carcinogen-induced activation of silent histocompatibility genes normally expressed in allogeneic strains should be considered, as suggested for other systems^{12,13}. In this case one would expect

to find a perfect identity between TATA and a given foreign histocompatibility antigen.

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Antibody to a molecularly-defined antigen confined to a tumour cell surface

TUMOUR-SPECIFIC antigens have usually been demonstrated with little knowledge of their molecular nature; for example by the residual reactivity with tumour shown by an anti-tumour serum which has been absorbed with normal tissue. Certain tumours of B lymphoid cells which secrete immunoglobulin (Ig) provide a promising situation for identification of the antigen in molecular terms¹⁻³. Amino acid sequences in the variable (V) regions of Ig molecules determine not only antibody activity, but also the 'idiotypic' antigenic determinants of the molecule (for review see ref. 4). In a normal B lymphoid clone the V regions on the surface Ig of the early members are the same as those on the Ig exported by specialised protein-secreting descendants and are essentially unique to this clone^{5,6}. The morphology and mode of Ig secretion in neoplastic cells are frozen at some stage in the range shown by their normally differentiating counterparts (Fig. 1). Tumours with cells having both surface and export Ig have provided Ig in the host serum in amounts suitable for the conventional raising of anti-V sera. These sera, by reacting specifically with the surface Ig of the corresponding tumour, demonstrated that the V regions can represent tumour-specific antigens^{2,3}. Furthermore, Lynch *et al.*¹ demonstrated that mice immunised so as to produce antibodies to the V regions of such a tumour (a syngeneic mouse myeloma) were thereby afforded a measure of specific protection against tumour challenge.

It is unlikely that anti-V sera raised in this way could be used therapeutically against the tumours concerned, because the exported Ig would represent a large extracellular barrier against access to the tumour cells. Consider, however, the therapeutically interesting case of neoplastic B lymphocytes having only surface Ig (Fig. 1). Are there definable idiotypic determinants on this surface Ig and is it possible routinely to raise the anti-V sera? Here we show that this is so in both cases for a guinea pig leukaemia.

The L₂C leukaemia of strain 2 guinea pigs is a transplantable tumour, maintained by repeated passage *in vivo*, composed of large B lymphocytes⁸ with scanty cytoplasm and no significant endoplasmic reticulum. They bear IgM on their surfaces, approximately 100,000 molecules per cell being shed with a half life of about 5 h (ref. 9). There is no evidence of IgM being delivered to extracellular fluid by any mechanism other than turnover on the cell surface⁹.

Blood was collected by exsanguination of animals near

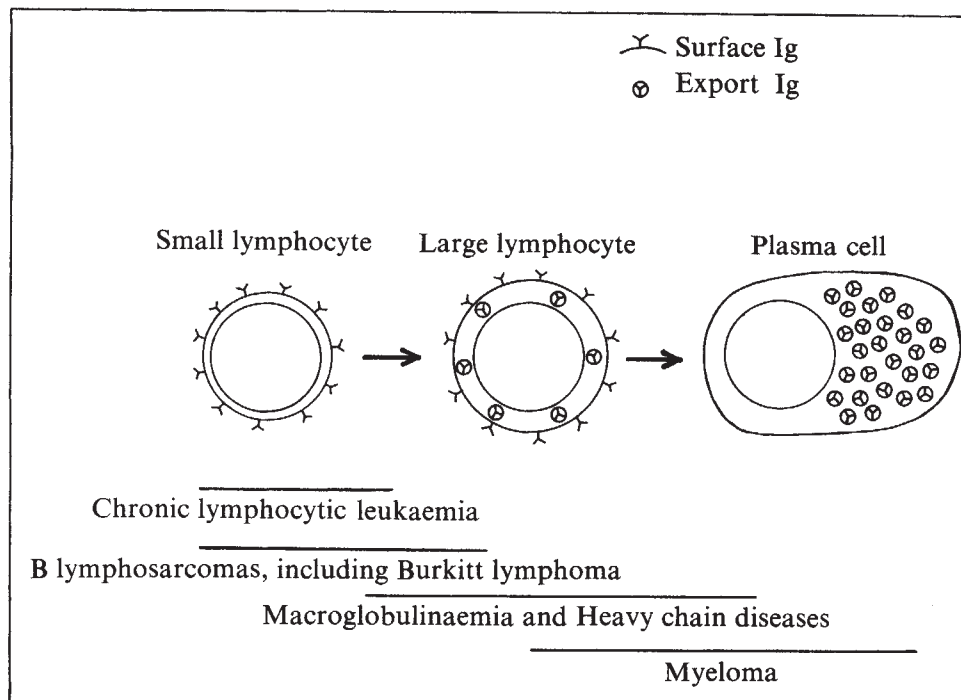


Fig. 1 Three morphological cell types distinguishable in the continuum of a normal maturing B lymphoid clone, with the associated modes of Ig secretion. Note that some cells exhibit both surface and export Ig (ref. 7). Neoplasms can seemingly arise at various stages to give clones in which differentiation is frozen. The horizontal lines depict the ranges of cell types commonly seen among the indicated neoplasms. The scheme is drawn for human neoplasms but is probably applicable broadly to all mammals.

death as a result of leukaemia, and the leukaemic lymphocytes (2×10^5 – $3 \times 10^5 \mu\text{l}^{-1}$) were separated and washed thoroughly. Limited proteolysis of the cells with papain^{9,10} (0.06 mg ml^{-1} acting on 10^8 cells ml^{-1} for 30 min at 37°C) cleaved the surface IgM *in situ* and released Fab μ fragments, bearing the V regions, into the supernatant. From here the Fab μ was isolated by binding to a microcrystalline cellulose immunosorbent, with an approximate yield of $75 \mu\text{g}$ per 10^{10} cells⁹.

Antibody to the V regions was raised by injecting the washed, Fab μ -laden immunosorbent from 3×10^{10} cells directly into two sheep (ref. 11 and Fig. 2). It should be noted that the schedule exposes the animals to particles on which the only foreign molecules are the cellulose matrix and the guinea pig Fab μ . The Fab μ contains V and constant (C) regions, so passive antibody to the C regions was injected simultaneously with the antigen to promote the anti-V response and depress the anti-C¹¹⁻¹³.

Serum was obtained from the sheep 1–3 weeks after the second injection of antigen. It contained low levels of two unwanted antibody activities: to the Fab μ C regions, judged by precipitin reaction, and to guinea pig normal cell surfaces

judged by immunofluorescent staining of normal leukocytes. These activities were removed by passing IgG from the sera through normal strain 2 guinea pigs. IgG from sheep normal serum was passed similarly to provide a control. The entire schedule (summarised in Fig. 2), provided us with guinea pig serum containing sheep IgG, antibody or control, at approximately 2 mg ml^{-1} . The 'antibody serum' and 'control serum' were tested for reactivity against L₂C cells and syngeneic normal lymphocytes (from lymph nodes, spleen and thymus) by immunofluorescence and inhibition of migration.

Immunofluorescence was by the indirect technique with all manipulations carried out at 4°C to prevent capping¹⁴. Cells at $2 \times 10^7 \text{ ml}^{-1}$ in Eagle's MEM were mixed with an equal volume of antibody or control serum and incubated for 30 min. They were then washed, treated with indicator reagent (fluorescein-conjugated IgG from rabbit antiserum to sheep IgG), washed again and examined in suspension. The only positive reaction observed was with antibody serum and L₂C cells, which showed uniform circumferential staining with superimposed bright spots.

Inhibition of migration was carried out as described by

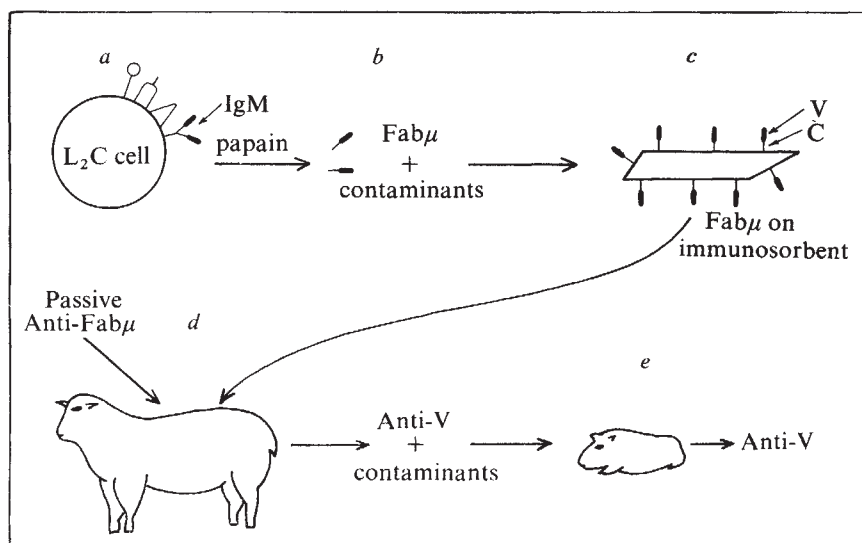


Fig. 2 Preparation of antibody to idiotypic determinants on V regions of surface Ig. *a*, L₂C cells contain many surface antigens among which the Ig is a trace constituent. *b*, Before exposure to the definitive immunosorbent the cellular supernatant undergoes a thorough preliminary absorption with a blank immunosorbent consisting of sheep normal IgG coupled to microcrystalline cellulose, thereby removing material adhering nonspecifically to the cellulose matrix. *c*, The definitive immunosorbent was prepared by coupling purified sheep anti-guinea pig Fab γ to the cellulose¹¹. *d*, Fab μ -laden immunosorbent was mixed with Freund's complete adjuvant and injected, in two doses five weeks apart, at multiple subcutaneous sites. Each dose was accompanied by intravenous administration of 30 ml sheep antiserum to guinea pig serum Fab μ , in effect an antiserum to the C regions of Fab μ . *e*, 100 mg IgG from unabsorbed sheep antiserum per 300-g guinea pig. The animals were exsanguinated 6 h later, each yielding approximately 20 mg sheep IgG in 10 ml serum.

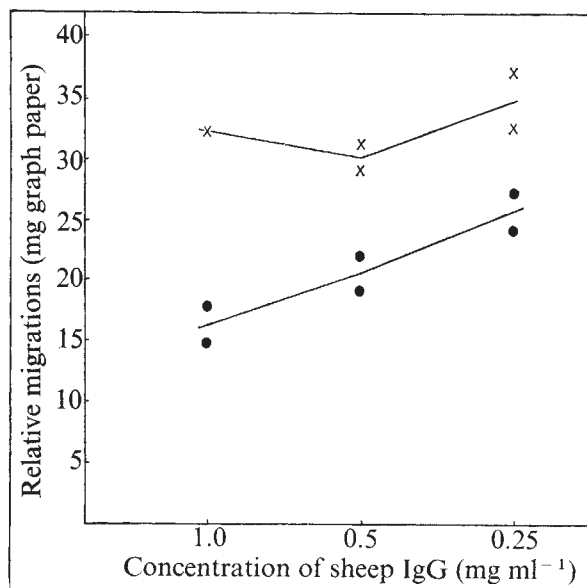


Fig. 3 Migrations of L_2C cells into nutrient medium containing sheep IgG passed through normal guinea pigs. $\times$, Normal IgG; $\bullet$, IgG from anti-V serum. Note that a concentration of sheep IgG of 1 mg ml^{-1} is equivalent to approximately a 1 in 20 dilution of a sheep serum. Migrations by normal (nodal and splenic) lymphocytes in the presence of normal or anti-V-containing IgG were indistinguishable.

Currie and Sime¹⁵. Cells at $5 \times 10^7 \text{ ml}^{-1}$ in MEM were taken into capillaries which were then sealed at one end with wax. After centrifuging the cells towards the seal each capillary was cut at the cell-medium interface, and the open end of the cell-containing part was attached with silicone grease to the base of a culture well in a Sterilin S25 plastic plate. The wells were filled with antibody or control serum and sealed with cover slips. Migration into the medium took place for 16 h at 37°C . Relative areas of migration were estimated by projecting the cell patterns on to paper, cutting round the edges and weighing. The results (Fig. 3) reveal a significant degree of inhibition by the antibody serum.

To confirm that the tumour-specific antibody is in fact directed against cell surface Ig, we took advantage of the molecular discrimination of the capping-pinocytosis phenomenon¹⁴, whereby two sets of antigenic determinants cap simultaneously if on the same molecule, but otherwise independently. In this case, antibody known to react with the surface Ig (in fact with C region determinants on the light chain) capped the determinants with which the tumour-specific antibody reacted and vice versa. For the first experiment L_2C cells were suspended at $2 \times 10^7 \text{ ml}^{-1}$ in rabbit anti-guinea pig Fab γ serum, 50% v/v in Eagle's MEM, and incubated at 37°C for 30 min. At the end of this period capping and internalisation were essentially complete, as indicated by the behaviour of an aliquot of cells allowed to react with fluorescein-conjugated IgG from the same antiserum. The cells were chilled and washed, and without being rewarmed, were shown to have lost virtually all their reactivity with the tumour-specific antibody serum in the indirect immunofluorescence test. In contrast, a sheep anti-guinea pig lymphocyte serum retained full reactivity with the treated cells. For the second experiment it was first found that incubation in tumour-specific antibody serum failed to give any capping on the L_2C cells, presumably as a result of the low antibody concentration in this serum. A second layer of anti-antibody on the cell could, however, induce capping: the cells were allowed to react at 4°C for 30 min with antibody serum and then, after washing, were incubated at 37°C for 30 min with a rabbit anti-sheep IgG serum which had previously been absorbed with guinea pig Ig. Again examination of an aliquot of cells treated in the second incubation with a fluorescein conjugate of the anti-antibody suggested that

capping and internalisation were essentially complete at the end of the 30-min period. The chilled, washed cells now showed greatly reduced reactivity with fluorescein-conjugated IgG from rabbit anti-guinea pig Fab γ . Cells treated similarly with control serum for the first step, showed the usual bright speckled fluorescence with fluorescein-anti-Fab γ .

We suggest that the availability of antisera to a tumour-specific molecule which can be identified and quantified, and which appears in the cellular environs simply as a result of turnover at the cell surface, is of considerable research and therapeutic interest. An approach similar to that used here may be appropriate for a variety of tumour-specific antigens.

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Thymus reactive IgM autoantibodies in normal mouse sera

AN important characteristic of the immune system is the ability to discriminate between antigens expressed on normal tissues within the individual and the many foreign antigens expressed on normal tissues of other species (xenoantigens) and even on normal tissues of members of the same species (alloantigens). Thus, although the immune system readily destroys inoculated xenogeneic and allogeneic tissue, autoimmune damage to normal, autologous tissue is rare. The rejection of inoculated foreign tissue is thought to reflect an immune surveillance mechanism by which an individual could detect and eliminate his own aberrant cells expressing abnormal surface antigens¹. This mechanism may be important in restricting the development of many nascent tumours¹⁻³. Little is known, however, concerning either the mechanism by which the immune system destroys nascent tumours or the mechanism preventing the immune system from inflicting autoimmune disease. The finding of near normal tumour incidence in congenitally athymic (nude) mice has suggested that immune surveillance may not involve the activity of thymus-dependent immunity but rather be a function of humoral immunity⁴. In this respect it is interesting that sera of both normal and nude mice contain antibodies reactive with a wide variety of tumour cells^{5,6}. There is no evidence, however, that these antibodies function in the destruction of nascent tumours or that they are necessarily directed against tumour specific rather than normal tissue associated antigens. It has been demonstrated that naturally occurring antibodies (NOA) reactive with the neural tumour cell line NBI can be specifically absorbed by syngeneic normal brain tissue⁶. We demonstrate further the autoantibody nature of certain NOA by documenting the occurrence in normal mouse sera of IgM antibodies reactive with autologous thymus cells.

Sera of C3H/HeJcrf mice (C3H) were tested in the trypan

blue dye exclusion cytotoxicity assay for complement dependent cytotoxic activity against syngeneic thymus and spleen cells. In 9 out of 10 experiments, serum of normal C3H mice caused significant lysis of syngeneic thymus cells (Table 1). In 12 experiments, the percentage lysis of syngeneic spleen cells by normal C3H serum varied from -5.4% to 7.3% with a mean specific lysis of -2.6%. The anti-thymus cytotoxic activity of normal C3H serum was rabbit complement (RC') dependent since lysis of thymus cells was not observed if the rabbit serum used in the assay had been heat inactivated at 56 °C for 30 min or if guinea pig serum, adequate for the detection of anti- θ^{C3H} antibodies, was substituted for rabbit serum as a source of complement. The anti-thymus cytotoxic activity of normal C3H serum was attributed to naturally occurring IgM antibodies since the cytotoxicity could be absorbed from mouse serum with anti-mouse immunoglobulin antibody-bound sepharose beads, and by a monospecific preparation of goat anti-mouse IgM antibody. The cytotoxic activity was heat labile and susceptible to 0.1 M 2-mercaptoethanol. In addition, when mouse serum was chromatographed on a Sephadex G-200 column, RC' dependent cytotoxic activity was detectable only in the excluded fraction (Table 2).

Undiluted normal sera of various strains were tested for RC' dependent cytotoxicity against autologous thymus cells. Sera of all strains tested were cytotoxic for autologous thymus cells (Table 3). Repeated experiments on mice obtained from

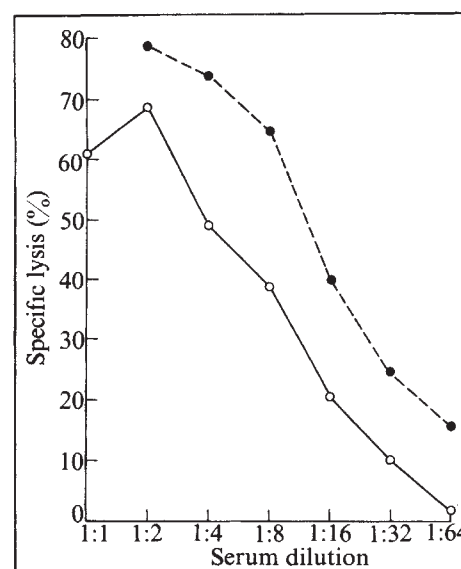


Fig. 1 Complement dependent anti-thymus cytotoxicity of sera obtained from nude (●) and normal C3H (○) mice. The titration curve for nude and normal mouse sera represents the mean of values obtained using three distinct pools of sera from both nude and normal mice.

Table 1 Complement dependent cytotoxicity of serum of C3H mice against syngeneic thymus cells

Experiment no.	Lysis resulting from RC' (%)	Specific lysis (%) resulting from normal serum plus RC'
1	2.3	38.9
2	6.2	26.2
3	8.3	6.3
4	5.7	47.0
5	12.8	52.8
6	4.7	24.6
7	3.7	36.4
8	8.5	39.5
9	3.2	23.7
10	4.1	22.9

Mice 3-6 months old were bled from the tail vein. The serum was separated by centrifugation approximately 1 h later and either tested directly or stored in aliquots at -20 °C. Cell suspensions were prepared by gentle teasing of the thymuses in Dulbecco's modified Eagle's MEM containing 10% foetal calf serum (DMEM-10) followed by filtration through 100-mesh nylon net. In the trypan blue dye exclusion cytotoxicity assay, 50 µl of a suspension containing 3×10^6 cells per ml in DMEM-10 were added to 50 µl of normal mouse serum. The mixture was incubated for 60 min in a humidified incubator at 37 °C in a CO₂-air (10:90) atmosphere. The cells were washed with 300 µl of medium and resuspended in 50 µl of the optimal dilution of rabbit serum as a source of complement (usually a 1:4 dilution). The rabbit serum had been absorbed previously at 2 °C with a one-third volume of packed thymus and spleen cells to reduce its toxicity. Several batches of rabbit serum were used in these experiments. Sera from some rabbits were excluded, however, because of either residual toxicity or low complement activity. After a 60-min incubation at 37 °C, tubes containing the cells and RC' were placed in an ice bath. A volume of 50 µl of trypan blue solution was added to the cell suspension and a sample was removed for microscopic enumeration of the percentage non-viable (stained) cells. At least 100 cells were counted in each determination. Controls included in each experiment were (1) incubation of cells with normal mouse serum followed by the addition of medium rather than RC' (antibody control), and (2) initial incubation of the cells in medium followed by incubation in RC' (RC' control). The antibody control value was invariably < 5%. The RC' control value is indicated in the table. In other experiments, unless otherwise indicated, the RC' control value was < 10%. Duplicate determinations were performed. The correlation observed between duplicate determinations was such that a calculated value of > 10% specific lysis was significant. The 10 sera used in the experiments presented in this table were obtained from groups of 3-5 mice, 2-6 months old. Specific, mouse serum induced lysis was calculated by:

$$\frac{(\% \text{ Cytotoxicity (mouse serum + RC') - RC' control}) \times 100}{100 - \text{RC' control}}$$

the Imperial Cancer Research Fund and from the Rodent and Rabbit Production Section of the National Institutes of Health have confirmed that sera of many mouse strains regularly (> 80% incidence) contain readily detectable levels of thymus reactive cytotoxic autoantibodies. Mouse sera are generally cytotoxic for allogeneic thymus cells, although thymus cells from different strains vary in their susceptibility to lysis by a given allogeneic serum (Table 3). Sera from nude mice possess significant levels of NOA reactive with allogeneic thymus cells (Fig. 1). To determine whether susceptibility to

Table 2 Evidence for IgM antibody nature of naturally occurring anti-thymus cytotoxic activity in normal C3H serum

Experiment no.	Serum treatment	Specific (%) lysis resulting from normal C3H serum plus RC'	
		Control serum	Treated serum
1	Heated (60 °C)	38.5	8.8
2	2-ME	33.0	2.2
3	Incubated with GAM IgM*	23.7	0.3
4	Absorbed with Seph-RAMi†	56.3	12.2
5	Chromatographed on Sephadex G-200‡	73.1	
	Unfractionated		
	1st peak (IgM)		79.2
	2nd peak (IgG)		3.4

The trypan blue dye exclusion cytotoxicity assay was performed and the specific (%) lysis calculated as described in the footnote to Table 1. Equal volumes of normal mouse serum and 0.2 M 2ME in phosphate-buffered saline were incubated at 37 °C for 45 min. Control serum was diluted with phosphate-buffered saline and similarly incubated.

*Monospecific goat anti-mouse IgM antibody was provided by Dr M. Cooper. 500 µg of anti-mouse IgM antibody in 100 µl balanced salt solution (BSS, pH 7.2) was mixed with an equal volume of normal mouse serum and incubated at 25 °C for 3 h. Normal mouse serum diluted with an equal volume of BSS served as a control.

†Sephadex-bound purified rabbit anti-mouse immunoglobulin antibody (Seph-RAMi) was provided by Dr D. Kilburn. Normal serum was incubated with either Sephadex or Seph-RAMi and incubated at 25 °C for 1 h. After centrifugation sera were tested for anti-thymus cytotoxic activity.

‡2 ml of serum was applied to a Sephadex G-200 column. Protein contained in the void volume (1st peak) and the 2nd peak was concentrated to 2 ml by Amicon filtration and shown by immunoelectrophoresis to react specifically with rabbit anti-mouse IgM and rabbit anti-mouse IgG antisera, respectively.

Table 3 Complement dependent cytotoxicity of normal mouse sera against autologous and allogeneic thymus cells

Mouse strain	Lysis resulting from RC' (%)	Specific (%) lysis resulting from autologous serum plus RC'	Specific (%) lysis resulting from normal C3H serum plus RC'
C3H	1.0	22.9	36.0
C57BL	1.0	29.2	46.3
A	2.5	20.3	29.1
BDF ₁	1.3	30.7	26.0
Hairless	17.9	21.0	16.8
DBA/2	4.4	50.0	ND
129	23.2	74.2	ND
Schneider	19.9	52.8	ND
NZB	12.6	76.3	ND
C57BT	9.2	31.6	ND

ND, not determined.

lysis by NOA was confined to functionally immature thymocytes, normal sera of C3H mice were tested for cytotoxicity against thymocytes obtained either from hydrocortisone-treated adult mice or from neonatal mice. As Table 4 shows, thymus cells of hydrocortisone-treated mice were susceptible to lysis by NOA. Thymus cells obtained from neonatal mice were lysed but only by undiluted normal serum.

Schlesinger found thymus reactive autoantibodies in sera of all strain 129 mice 5 weeks or older⁸. Shirai and Mellors, however, detected thymus reactive antibodies in only 46 out of 120 strain 129 mice 2–11 months old⁹. In a survey of 10 other strains Shirai and Mellors regularly detected antibodies reactive at 37 °C with thymocytes in sera of 2–11 month old mice of the NZB strain (69 out of 97 sera tested). Anti-thymus antibodies were detected in a small proportion of normal sera obtained from several other strains, including C58 (17 out of 40 sera tested), and NZW (6 out of 25 sera tested) and were occasionally present in sera of AKR (14 out of 120), BALB/c (1 out of 29), C3HeB/Fe (3 out of 30), C57BL/6 (2 out of 90) and SJL (1 out of 21) strain mice. Thymus reactive cytotoxic antibodies were not detected in 23 sera of DBA/2 mice or in 20 sera of A mice. Boyse *et al.*¹⁰ detected thymus reactive autoantibodies in several mouse strains but only after the mice were immunised with allogeneic thymus cells. These reports stand in contrast to that of Raff who, using hamster serum as the source of complement, detected high titres of thymus reactive antibodies in sera of all strains tested¹¹. Our findings confirm and extend

Table 4 Reactivity of normal serum of C3H mice against thymus cells of hydrocortisone-treated adult and untreated neonatal C3H mice

Thymus cell donor	Specific (%) lysis resulting from 1:1 and 1:2 dilutions of C3H mouse serum plus RC'	
	1:1	1:2
Normal adult	58.6	64.4
Hydrocortisone-treated adult*	41.7	49.4
Normal neonatal†	33.8	—2.9

*Six week old mice received 5 mg hydrocortisone acetate intraperitoneally 48 h before removal of their thymus. Less than 10⁷ thymocytes could be obtained from each hydrocortisone-treated mouse, whereas approximately 10⁸ thymocytes were obtained from each normal mouse.

†Mice less than 24 h old were used as thymus donors.

those of Raff. The failure of many investigators to detect thymus reactive autoantibodies in normal mouse sera may be the result of their use of inadequate amounts of complement or of complement with a high level of residual cytotoxicity due to inadequate absorption with normal tissue. Thus, for example, when RC' was used at a lower than optimal concentration cytotoxic anti-thymus antibodies were detected in sera of 129 and NZB mice but not in sera of C3H, C57BL or A mice (data not shown).

The finding of thymus reactive antibodies in sera of nude

mice indicates that the thymus is not required as the antigenic source for the production of such antibodies and that the antibody response in normal mice is probably thymus independent. Absorption studies have indicated that several thymus antigens are recognised by NOA. The nature and tissue distribution of individual antigens are being analysed. The observation that hydrocortisone-resistant thymus cells are susceptible to lysis by NOA indicates that even within the thymus many of these antigens are not restricted to functionally immature thymocytes. Whether the cells which actually leave the thymus continue to express these antigens has not been confirmed. In any event the injection of thymocytes directly into the circulation provides an experimental system with which to study the consequence of the interaction of NOA and target cell and may help to elucidate the biological function of tumour reactive NOA.

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Carrier preimmunisation in the anti-hapten response of a marine fish

A STRONG antibody response in mammals and birds requires collaboration of two types of antigen-recognising lymphocytes, T and B. Only the B lymphocyte gives rise to antibody-producing plasma cells. This concept is partly based on the observed synergistic effect of thymic (non-B) cells and bone marrow (non-T) cells in antibody responses^{1–3}. Another line of evidence comes from immunisation with an antigen having several antigenic determinants such as A–B–C–D, which can be separated into portions. A strong synergistic effect in an antibody response against A is found by combining lymphocytes primed with one part of the antigen (B–C–D = carrier) with lymphocytes primed with another part (A = hapten) and challenging the mixture with A–B–C–D (refs 4 and 5). T lymphocytes seem to be the relevant cells in the carrier-primed population⁶.

Whether the collaboration of two types of antigen-recognising lymphocytes is also important for antibody responses of lower vertebrates is not known. Studies of fishes, amphibia and reptiles are handicapped by the lack of histocompatible strains. This excludes cell transfers from one individual into another, which have been the basis of all the studies cited above and makes *in vitro* collaboration experiments in lower vertebrates unattractive since histocompatibility between collaborating cells may be necessary for a good response *in vitro*⁷. A phenomenon which can strongly suggest importance of cell collaboration and does not involve cells from more than one individual animal is an enhanced anti-hapten response caused by carrier preimmunisation. This has been shown to correlate well with cell transfer data in mammals and birds^{8–11}, and we have used this method in the winter flounder (*Pseudopleuronectes americanus*).

Winter flounder, weighing between 0.2 and 0.3 kg, were caught using an otter trawl in Sandy Hook Bay, and were

Table 1 Anti-NIP response in the winter flounder

Group	No. of fish	Priming	Challenge	Log mean anti-NIP titre $\pm$ s.e.
A	9	CG-FCA*	NIP ₁₂ CG†	5.534 $\pm$ 0.190
B	7	Saline-FCA	NIP ₁₂ CG	4.344 $\pm$ 0.163
C	16	None	None	4.435 $\pm$ 0.062

*Fishes were primed with 1 mg kg⁻¹ chicken globulin (CG) in Freund's complete adjuvant (FCA).

†4-Hydroxy-3-iodo-5-nitrophenyl acetyl conjugated to CG (1 mol CG to 12 mol NIP 0.5 mg kg⁻¹ in FCA).

‡Haptenated phage inactivation titre on day 21.

P values for A against B or A against C: < 0.001.

Difference between B and C was statistically insignificant.

kept in continuous flow salt water tanks in the range 16–18 °C. The fishes were injected intraperitoneally with chicken globulin (1 mg per kg of body weight) in complete Freund's complete adjuvant (FCA); controls were given saline in FCA. After 3 weeks both groups were injected with 0.5 mg kg⁻¹ NIP-CG (Table 1) and bled for sera after 3 weeks by puncture of the dorsal aorta under anaesthesia with triclanemethane sulphate. The antibody was detected by the haptenated phage inactivation test according to the method of Becker and Makela¹².

The results indicate that one injection of the conjugate did not cause a measurable anti-NIP response in 3 weeks unless the animals were preimmunised with the carrier protein (Table 1). Flounders which had been preimmunised by FCA alone had antibody titres indistinguishable from the level of natural anti-NIP in this species. Carrier-preimmunised animals, on the other hand, had more than ten times greater titres than either of the other groups.

Flounder sera were unexpectedly strong inactivators of NIP-T4 phage. This inactivation may be caused by natural anti-NIP antibodies. Flounder sera did not inactivate unconjugated T4 phage, and inactivation of the NIP-T4 was inhibited by 3 $\times$ 10⁻⁶ M NIP but not by 10⁻³ M DNP (highest tested concentration). Both haptens were in the form of protein conjugates (10–15 mol hapten per mol BSA).

Our finding suggests that in fishes carrier-recognising cells also collaborate with hapten-specific cells for an optimal anti-hapten response. As fishes are the most primitive major order of antibody forming animals, the available data suggest that collaboration of two types of recognising lymphocytes is a general requirement for the initiation of antibody production.

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Symmetrical transcription of herpes simplex virus DNA in infected BSC-1 cells

ALONI^{1–3} reported that late in the replicative cycle of polyoma and simian virus 40 (SV40) viruses the viral DNA genomes are symmetrically transcribed in the infected cells. The newly synthesised RNA molecules labelled for very short periods were shown to be complementary and on self-annealing yielded high molecular weight double stranded (ds) RNA. Aloni and Attardi showed that similar symmetrical transcription occurs in HeLa cell mitochondria^{4,5}. In further studies Aloni² demonstrated that after polyadenylation of the SV40 symmetrical RNA transcripts the RNA is processed to yield uncomplementary viral mRNA molecules.

Since uninfected BSC-1 green monkey kidney cells which were used in our studies on herpes simplex virus (HSV) were found by Aloni¹ to have very small amounts of self-annealing RNA, we decided to study the nature of the transcription of HSV DNA, a linear genome with a molecular weight of 100 $\times$ 10⁶ (ref. 6). Studies by Roizman and his collaborators on the transcription of HSV DNA in infected nuclei revealed the synthesis of a high molecular weight precursor to viral mRNA^{7,8} and the presence of polyadenylic acid [poly(A)] sequences in some of the virus-specified RNA⁹. Wagner *et al.*¹⁰ reported that 90% of the HSV DNA genomes are transcribed late in the infection. There is however no information on the mode of transcription and the DNA strand selection for transcription of HSV DNA. Here we demonstrate that viral mRNA molecules which lack poly(A), transcribed from HSV DNA in BSC-1 cells late in infection, contain complementary sequences.

BSC-1 monolayer cultures were infected with the HF strain of herpes simplex virus type 1 (ref. 11). In these cells the HSV DNA is synthesised from 3 to 15 h after infection and the virus growth cycle is completed at 18 h. The infected cells were pulse labelled with ³H-uridine at 12 or 13 h after infection for 15 to 20 min, the RNA was extracted and analysed for complementary sequences by self annealing. The amount of HSV specific RNA in the total RNA isolates does not exceed 10% as determined by hybridisation with HSV DNA.

Figure 1a demonstrates the isolation of dsRNA molecules after self-annealing of labelled RNA. HSV infected cells were labelled for 15 min at 13 h after infection and the total labelled

Table 1 Nuclease resistance of self-annealed RNA*

Enzyme treatment	Trichloroacetic acid precipitable		
	³ H-self-annealed RNA	³ H-rRNA	³ H-HSV DNA
None	100	100	100
Pancreatic RNase	100	3	ND
Pancreatic RNase + DNase	100	ND	5
Denatured RNA or DNA with RNase or DNase	5	5	12

*Total RNA from infected cells was prepared as indicated in Fig. 1b. Self-annealing was done in 4 $\times$ SSC at 72 °C for 17 h and the self-annealed RNA peak was eluted from the Sephadex G-50 column after RNase (25 μ g ml⁻¹) treatment. RNase treatment was in 0.2 M NaCl, 10 mM Tris-HCl, pH 7.4, 10 mM EDTA at 37 °C for 60 min with 2,000 c.p.m. of self-annealed RNA or 2,000 c.p.m. ribosomal RNA (rRNA). DNase treatment was with 2,000 c.p.m. HSV DNA in 100 mM NaCl, 10 mM MgCl₂, 10 mM Tris-HCl pH 7.4 at 37 °C for 60 min. For the simultaneous incubation of RNase and DNase, 10 mM MgCl₂ (final concentration) was added to the RNase buffer without EDTA. Thermal denaturation was at 100 °C for 5 min after which the samples were cooled in ice water. The trichloroacetic acid (TCA) precipitable material was determined.

ND, not done.

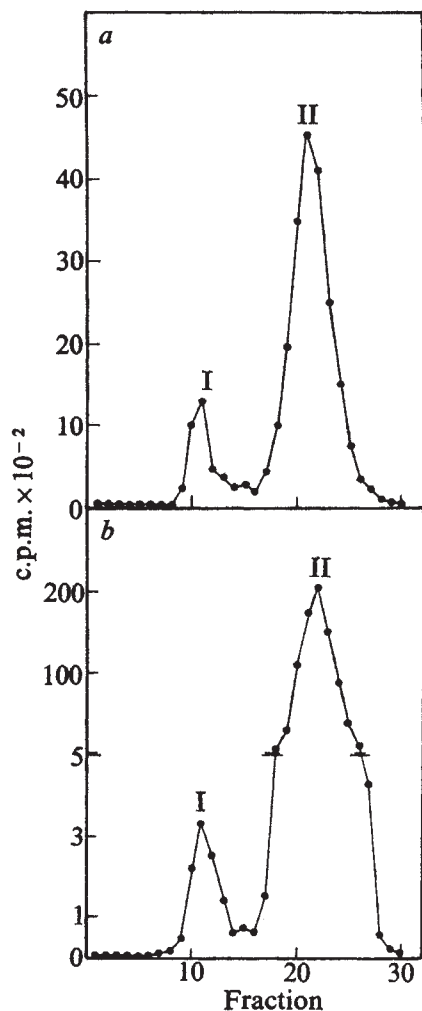


Fig. 1 Exclusion chromatography of self-annealed, RNase-treated RNA on a column of Sephadex G-50. *a*, Total RNA; 1.5×10^7 BSC-1 cells were infected with HSV (10 PFU per cell) and incubated at 37°C for 13 h. The infected cell cultures were then labelled with ^3H -uridine for 15 min ($200 \mu\text{Ci ml}^{-1}$; specific activity 29 Ci mM^{-1}). The cells were chilled in ice at the end of the labelling period and scraped into RSB buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 1.5 mM MgCl_2). The swollen cells were homogenised in a tight glass Dounce homogeniser (30 strokes). Total labelled RNA was extracted twice with a mixture of phenol and chloroform 1 : 1 (v/v) at room temperature and twice with chloroform-isoamyl alcohol (99 : 1). The RNA was precipitated in ethanol, dissolved in DNase buffer (10 mM Tris-HCl pH 7.6, 100 mM NaCl, 10 mM MgCl_2) and incubated with DNase ($50 \mu\text{g ml}^{-1}$) for 15 min at 37°C . The RNA was extracted once more as above and was brought to 60% formamide and $2 \times \text{SSC}$ ($1 \times \text{SSC} = 0.15 \text{ M NaCl}$, 0.01 M sodium citrate) and annealed by incubation at 37°C for 24 h. The RNA was precipitated with ethanol and resuspended in RNase buffer (0.2 M NaCl, 10 mM Tris-HCl pH 7.6 and 10 mM EDTA) and treated with RNase ($5 \mu\text{g ml}^{-1}$) at 37°C for 60 min. The RNA was diluted with the application buffer (0.1 M NaCl, 0.1% sodium dodecyl sulphate (SDS), 2 mM EDTA, 10 mM Tris-HCl, pH 7.7) and applied to a 100 cm Sephadex G-50 column. Thirty fractions of 2.5 ml each were collected and 25 μl samples were used to determine the radioactivity in each fraction. Peak (I) is the self-annealed RNA and peak (II) contains the low molecular weight RNA products, which remain after RNase treatment. *b*, Exclusion chromatography of self-annealed, RNase treated RNA which did not bind to a cellulose column (poly(A) minus RNA). HSV infected cells at 12 h after infection were pulse-labelled for 15 min with ^3H -uridine ($200 \mu\text{Ci ml}^{-1}$). Total RNA was prepared as in *a*. After DNase treatment and phenol extraction, the aqueous phase was diluted with the application buffer containing 0.5 M KCl, 10 mM Tris-HCl pH 7.6 and 0.2 mM MgCl_2 . The solution was applied to a cellulose column using the technique of Schutz *et al.*¹². The RNA was washed with the application buffer (0.5 M KCl, 0.2 mM MgCl_2 , 0.01 M Tris-HCl pH 7.6) and the unbound RNA was eluted. After extensive washing with the buffer, when no more labelled RNA was eluted the cellulose

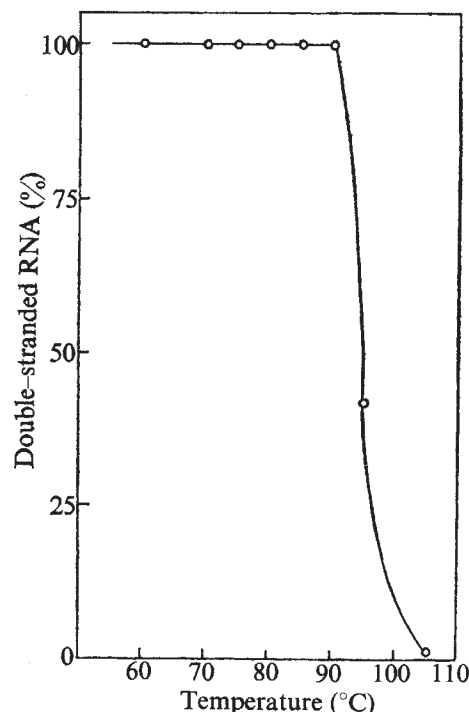


Fig. 2 RNase susceptibility of self-complementary RNA as a function of the denaturation temperature. HSV-infected BSC-1 cells were labelled with ^3H -uridine ($200 \mu\text{Ci ml}^{-1}$) for 20 min at 13 h after infection. Total RNA was extracted as described in the legend to Fig. 1*a* and self-annealed for 17 h at 72°C in $4 \times \text{SSC}$ buffer. The preparation was RNase treated (30% of the radioactivity was RNase resistant) and chromatographed on a Sephadex G-50 column as in Fig. 1*b*. The labelled RNA which was eluted in the first peak (as shown in Fig. 1) was ethanol precipitated overnight and resuspended in $0.1 \times \text{SSC}$. Samples (each containing 1,600 c.p.m.) from the dsRNA peak were distributed into glass tubes in $0.1 \times \text{SSC}$ and heated for 5 min at the indicated temperatures and rapidly cooled. The samples were then brought to $2 \times \text{SSC}$ and treated with $50 \mu\text{g ml}^{-1}$ pancreatic RNase for 60 min at 37°C . The samples were TCA precipitated and the radioactivity was determined.

Table 2 Hybridisation of denatured dsRNA with HSV DNA

^3H -RNA source	DNA source	Input (c.p.m.)	Hybridised (c.p.m.)	%
Denatured dsRNA	HSV	2,000	450	22.5
	BSC ₁	2,000	86	4.3
	—	2,000	75	3.7
Cellular RNA	HSV	50,000	450	0.9
	BSC ₁	50,000	1,600	3.2
	—	50,000	85	0.17

^3H -labelled dsRNA was obtained from the infected cells used for the experiment described in Fig. 1. The dsRNA was denatured by heating for 5 min at 100°C , cooled rapidly and hybridised in solution to $30 \mu\text{g ml}^{-1}$ of alkali-denatured HSV DNA or $350 \mu\text{g ml}^{-1}$ of alkali-denatured BSC₁ DNA. The HSV DNA was purified from herpes simplex virions isolated in sucrose gradients. The hybridisation was carried out in a buffer containing 0.4 M NaCl, 1 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, final volume 100 μl for 24 h at 72°C . At the end of the hybridisation each sample was treated with RNase ($50 \mu\text{g ml}^{-1}$ for 60 min at 37°C) and the hybrids were trapped on Millipore filters. The filters were washed with $2 \times \text{SSC}$ and the radioactivity determined.

column was washed with neutralised H_2O to elute the poly(A)-containing mRNA which was retained on the column. The unbound RNA species which were eluted with the application buffer were used for self-annealing in $4 \times \text{SSC}$ at 72°C for 17 h. The samples were treated with $25 \mu\text{g ml}^{-1}$ RNase in RNase buffer as in *a*. The RNA solution was chromatographed on a Sephadex G-50 column as in *a*. Peak I is the self-annealed RNA and peak II contains the low molecular weight RNA products, which remain after RNase treatment.

RNA was extracted. Similarly, dsRNA molecules were obtained from a ^3H -RNA preparation which was chromatographed on a cellulose column to isolate the poly(A) containing RNA molecules by the technique of Schutz *et al.*¹² In these conditions 12% of the total labelled RNA was bound to the column. About 27% of the labelled RNA molecules synthesised in the nuclei of HSV infected BSC-1 cells are complementary and can form double-stranded RNA molecules, resistant to RNase treatment. Further studies on the self-annealing of the poly(A) containing labelled RNA are in progress.

We studied further the properties of the dsRNA molecules. Heating as described in Fig. 1 resulted in the denaturation of the dsRNA molecules and rendered them sensitive to RNase treatment (Fig. 2). The dsRNA underwent a sharp transition in nuclease resistance at 95 °C in $0.1 \times \text{SSC}$; a higher temperature than SV40 dsRNA^{1,2}. The dsRNA was completely resistant to RNase and to a mixture of RNase and DNase at

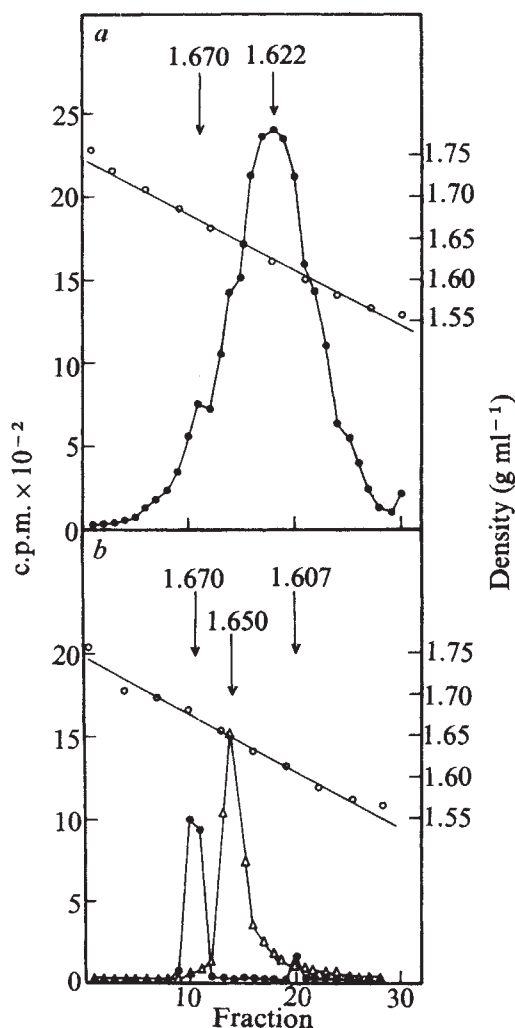


Fig. 3 Buoyant density of self-complementary RNA in Cs_2SO_4 gradients. HSV infected BSC-1 cells, at 12 h after infection were labelled with ^3H -uridine ($200 \mu\text{Ci ml}^{-1}$) for 15 min and the total RNA was extracted as described in the legend to Fig. 1a, self annealed RNase treated and eluted from a Sephadex G-50 column. The RNA preparation was divided into two portions. One was left untreated (a) and the second was heated (4 min at 100 °C) and quickly cooled (b). To the two ^3H -RNA preparations in buffer (0.05 M Tris-HCl, pH 7.4, 0.005 M EDTA), Cs_2SO_4 crystals were added to an initial density of 1.580 g ml^{-1} . To gradient (b) ^{14}C -labelled ribosomal RNA was added as a density marker. The tubes were centrifuged at 35,000 r.p.m. in the Beckman ultracentrifuge at 20 °C in the rotor 50 Ti for 46 h. The gradients were collected dropwise from the bottom and the density and the TCA precipitable radioactivity in each fraction were determined. Symbols: a, ●—● self-annealed dsRNA; ○—○ density. b, ●—● denatured self-annealed ^3H -RNA; !△—△ ^{14}C ribosomal RNA marker; ○—○ density.

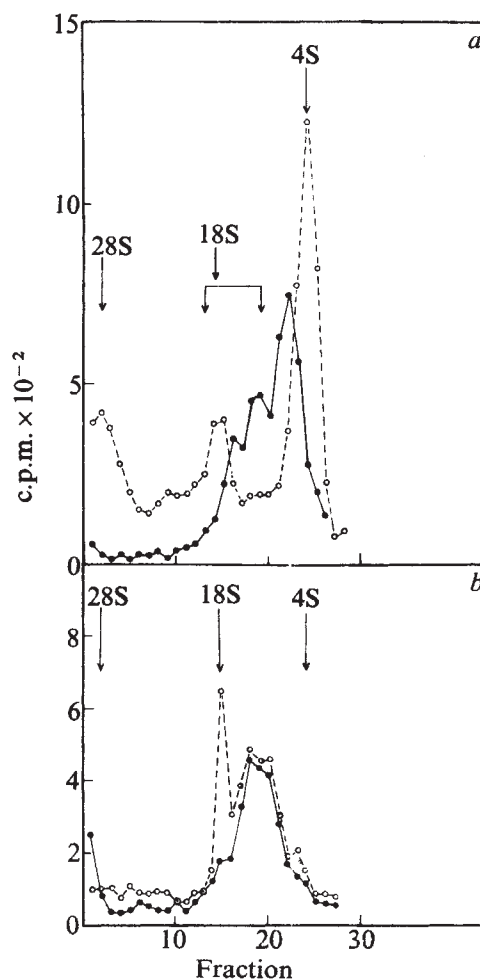


Fig. 4 Sedimentation of dsRNA in sucrose gradients before and after denaturation. a, dsRNA (●—●) was prepared as indicated in Fig. 1. The dsRNA resuspended in SDS buffer after Sephadex G-50 filtration was layered onto a linear 5–20 (w/w) sucrose gradient in RSB containing 0.5% (w/v) SDS and centrifuged in SW41 rotor at 40,000 r.p.m., at 20 °C for 4.5 h. Fractions were collected from the bottom of the tube dropwise and the TCA precipitable material was determined in each fraction. ^{14}C -labelled rRNA (○—○) served as internal marker. b, Fractions 13–19 from the gradient (a arrows) were pooled, ethanol precipitated and resuspended in 10 mM Tris-HCl pH 7.4, 1 mM EDTA and divided into two portions; one (○) was denatured with formaldehyde at 70 °C as described by Aloni¹, the other (●) was kept untreated. The samples were centrifuged in a 5–20 sucrose (w/w) gradient containing 0.5% (w/v) SDS in RSB in the SW41 rotor at 20 °C at 40,000 r.p.m. for 4.5 h. Fractions were collected dropwise and the TCA precipitable radioactivity was determined in each fraction, ^{14}C -uridine-labelled rRNA served as internal marker.

37 °C, but became completely sensitive to RNase after heating for 5 min at 100 °C (Table 1).

The labelled dsRNA molecules were centrifuged in Cs_2SO_4 and were found to band at a density of 1.622 g ml^{-1} (Fig. 3a) whereas after heat denaturation the RNA molecules banded at a density of 1.670 g ml^{-1} (Fig. 3b). The molecular size of the dsRNA molecules was determined by centrifugation in sucrose gradients (Fig. 4). Some of the radioactive RNA sedimented to the position of 4S and some ranged from 10S to 16S. Denatured RNA molecules also sedimented with a peak at approximately 18S, while the main mass sedimented at 8–10S (Fig. 4b).

To determine the specificity of the dsRNA in respect to its homology with HSV DNA, the dsRNA molecules were isolated, denatured and hybridised in solution with denatured HSV DNA. The dsRNA hybridised to HSV DNA and not to BSC-1 DNA (Table 2). It was noted that only 22.5% of the input RNA hybridised to HSV DNA. This value may indicate that the

efficiency of hybridisation is low and that not all the RNA was HSV specific.

Our study provides evidence that the primary transcription products in the nuclei of HSV-infected BSC-1 cells are transcribed from the two strands of the viral DNA in a symmetrical fashion. The excluded peak from the Sephadex column is dsRNA as indicated by first, complete resistance to RNase at 37 °C, second, complete resistance to the simultaneous action of RNase and DNase, third, the sharp transition curve in nuclease resistance at 95 °C in 0.1×SSC, which is not the melting temperature of the DNA-RNA hybrid, fourth, the density of 1.622 g ml⁻¹ of the dsRNA which differs from the density of DNA-RNA hybrids, and finally the transition to the high density of 1.670 g ml⁻¹, which is specific for G+C rich DNA, on heat denaturation of the dsRNA.

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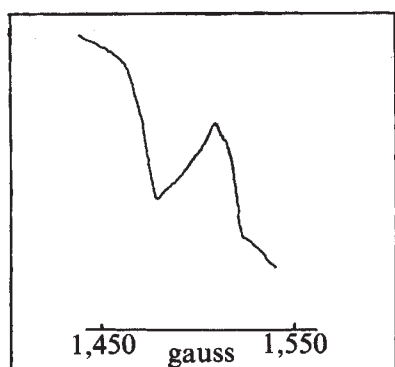
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Conversion of photoexcitation energy in rhodopsin

AN interaction between the 11-*cis*-retinylidene chromophore and nearest-neighbour molecules seems to be important for explaining the mechanism of conversion of photoexcitation energy, because the chromophore is enveloped by opsin. It has been reported¹ that the time of the formation of prelumi-rhodopsin (lifetime of the excited state) is less than 6 ps.

A weak electron spin resonance (ESR) signal for the forbidden transition $\Delta m = \pm 2$, as shown in Fig. 1, was observed following

Fig. 1 ESR spectrum ($\Delta m = \pm 2$) of rhodopsin irradiated with visible light at 77 K. The microwave frequency is about 9,230 MHz and the reproducibility of this ESR signal was about 70%. Clear experimental conditions for inducing the triplet state were not discovered. Free radicals ($g \approx 2.00$, $\Delta H \approx 18$ gauss) were also observed by ultraviolet irradiation (K.S., unpublished).



the irradiation with visible light ($\lambda = 450$ –600 nm) of frog rhodopsin extracted with 2% digitonin and mixed with an equal volume of glycerol so that it would freeze to a glass at 77 K. A set of glass filters was used which cut out any light of less than 450 nm. The intensity of illumination was $\sim 1,500$ lx at the window of the cavity. About one-tenth of the rhodopsin molecules (10^{14} – 10^{15} unbleached rhodopsin molecules per effective volume) was transformed into the triplet state by irradiation. This resonance signal disappeared after melting the irradiated rhodopsin and then freezing it again at 77 K. The solvent in the same glass tube without rhodopsin did not show such a resonance signal even after the same irradiation. The important factors for this signal are the zero-field splitting parameters (D and E), because the spin orientation in the external magnetic field and the microwave frequency are known parameters of the experiments. $D^* = (D^2 + 3E^2)^{1/2}$ for rhodopsin is estimated from the magnetic field (H_{min}) of this signal as 0.117 ± 0.002 cm⁻¹. The excitation of rhodopsin results in photochemical transformation of the 11-*cis*-retinylidene chromophore to all-*trans*², probably by way of the lowest triplet state T_1 (ref. 3). D for the retinal was evaluated by⁴

$$D = \frac{1}{2} g^2 \beta^2 \sum_{p < q} (C_{ip} C_{jq} - C_{iq} C_{jp})^2 \times \langle \chi_p(1) \chi_p(1) | r_{12}^{-2} - 3z_{12}^2/r_{12}^5 | \chi_q(2) \chi_q(2) \rangle \quad (1)$$

where β is Bohr magneton, C_{ip} and so on are the linear combination of atomic orbital (LCAO) coefficients, χ_p represents an atomic π orbital associated with the p th carbon atom, r_{12} is the distance from electron 1 to electron 2 and z is chosen parallel to the π orbitals of the retinal. From equation (1), it is expected that the Hückel (H) and self-consistent-field coefficients are of the same order. When D for the retinal was evaluated using the coefficient H , its value was about 0.057 cm⁻¹. E for the retinal is not zero, because of poor symmetry, but D^* for rhodopsin is greater than D^* for the retinal, so that E is generally chosen under the condition $|D| \geq 3|E|$ using the values of D (0.117 cm⁻¹ and 0.057 cm⁻¹) mentioned previously. The triplet state was not therefore, the result of the conversion of the chromophore during isomerisation of 11-*cis* into all-*trans*, but was assumed to have arisen from photoexcited energy transfer from the chromophore to a molecule having π electrons, for example an aromatic amino acid of opsin. The energy differences of the excited and the ground states of aromatic amino acids are,

Table 1 D^* values and linewidths, ΔH , of aromatic amino acids

Aromatic amino acids	$D^* = (D^2 + 3E^2)^{1/2}$ (cm ⁻¹)	ΔH (gauss)
L-Tyrosine		
Non-ionised	0.161†	} (18)
Ionised	0.141†	
Tryptophan	$0.117 \pm 0.001 \ddagger$	28
Tryptophyl-tyrosine	$0.119 \pm 0.001 \ddagger$	20
Phenylalanine	$0.154 \pm 0.001 \ddagger$	—
Rhodopsin	0.117 ± 0.002	30

†Ref. 6.

‡Calculated values from data of refs 7 and 8.

however, higher than those of the chromophore. In this system, the inference is that formation of a hydrogen bond between the chromophore and an amino acid is necessary for this energy transfer, that is a charge transfer. It may be suggested that the changes of λ_{max} of rhodopsin which is denatured by trichloroacetic acid⁵ are attributable to the hydrogen bond between the chromophore and the amino acid. The mechanism of the charge

transfer is probably induced by the electron transfer interaction between the π systems of the chromophore and of the amino acid. The σ - π interaction of the hydrogen bond, $=\dot{N}-H\cdots$, may be an exchange interaction, presumably involving the proton transfer. The wave function of the retinylidene chromophore may be written as

$$\Psi \approx C_1\phi_0 + C_2\phi_{CT} \quad (2)$$

where ϕ_0 and ϕ_{CT} mean the states containing the hydrogen bond and the charge transfer structure between the chromophore and the amino acid, respectively. The observed signal is not attributable to the intermolecular triplet state, because D for rhodopsin is large. We compared the D^* of aromatic amino acids and of rhodopsin (see Table 1); these results suggest that the ESR signal arises from the triplet state of tryptophan. Although the process (CT complex $\rightarrow$ triplet state of tryptophan) is not yet known, it is possible that the triplet state of the tryptophan arises by way of a metastable state because the lifetime of the excited state of rhodopsin is short¹.

The mechanism of the charge transfer seems to be an important clue in clarifying the problems of the early receptor potential⁹⁻¹⁰ which may be generated by charge separation in the rhodopsin molecule¹¹.

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NH₂-terminal amino acid sequence of the Fc fragment of IgD resembles IgE and IgG sequences

THE function of IgD, a rare immunoglobulin (Ig) class, is unknown¹. It has been shown that most B lymphocytes in cord blood² and lymphocytes of patients with chronic lymphatic leukaemia³ carry IgD on their surfaces, whereas only a small portion of lymphocytes from healthy human adults is IgD-positive⁴. Almost all lymphocytes having IgD on the surface also carry IgM and both seem to function as receptors⁵. To learn more about the relationship of IgD to other Ig classes, we are investigating the amino acid sequence of the IgD Fc fragment. This report describes our first sequence analysis which suggests that IgD is more closely related to IgE and IgG than to IgM.

The NH₂-terminal amino acid sequence of the Fc fragment is shown in Fig. 1. Two preparations of Fc fragments of protein Di and one of protein Ac were analysed for 42 steps and one preparation of Ac for 53 steps. All preparations showed a single sequence starting with the residue Thr, and the Fc fragments of proteins Di and Ac were alike. The first 13 amino

acids had a sequence identical to that reported for the peptide of the δ chain that contains the half-cystine residue forming the sole inter-heavy-heavy chain disulphide bond in IgD (ref. 8) allowing us to place the determined sequence at the beginning of the Fc fragment. The sequence in this area of the δ chain was characterised by a relatively high content of aromatic amino acids. One residue each of histidine, tyrosine and tryptophan was found in positions 7, 14 and 24, respectively, and two phenylalanine residues in positions 31 and 34. In addition, there was probably a tryptophan residue in position 52 and a tyrosine

1	10
THR - PRO - GLU - CYS - PRO - SER - HIS - THR - GLN - PRO -	
11	20
LEU - GLY - VAL - TYR - LEU - LEU - THR - PRO - ALA - VAL -	
21	30
GLN - ASP - LEU - TRP - LEU - ARG - ASX - LYS - ALA - THR -	
31	40
PHE - THR - (CYS) - PHE - VAL - VAL - GLY - THR - ASX - LEU -	
41	50
LYS - GLY - ALA - LEU - PRO - THR - ? - VAL - GLX - LEU -	
51	53
GLY - (TRP) - TYR	

Fig. 1 NH₂-terminal amino acid sequence of IgD Fc fragment of myeloma proteins Ac and Di. The Fc fragments were isolated after digestion of IgD with trypsin for 2 min⁶. We applied 300 to 600 nM into the cup of an updated Beckman Model 890B automated sequencer using the DMAA program No. 11374. The cleaved amino acids were identified either by gas chromatography or automated amino acid analysis after hydrolysis with 6 N HCl or HI as previously described⁷. The protein was not fully reduced and alkylated with ¹⁴C-iodoacetamide to determine half-cystine residues because such preparations were insoluble and unsuitable for sequence analysis. The half-cystine residue in position 4 was recovered as alanine after HI hydrolysis and the residue in position 33 was assumed to be a half-cystine because of the absence of any recoverable residue at this position and homology to the other Ig Fc fragments. The tryptophan residue at position 24 was identified by gas chromatography and as an increase of glycine after hydrolysis with HI. Position 52 was probably also tryptophan because there was no residue other than a slight increase in glycine after HI hydrolysis. No residue could be identified in position 47. The extrapolated initial yield of the Fc preparation (Ac) that could be sequenced for 53 steps was 296 nM, approximately 600 nM had been applied to the cup. The average loss per step determined during the first 20 steps was 3.8%. The yields over background after step 35 varied slightly from step to step and were decreasing gradually from 30 to 4 nM as in the case of tyrosine in step 53.

residue in position 53 because three other Ig classes have a tryptophan residue in the analogous position, and no other residue was detected in this position and at step 53 tyrosine was detected in about three times the background. The yields of amino acids after step 35 were low, and therefore the sequence after step 35 is less reliable. No residues could be identified in position 47. Although low yields in the late steps may have led to erroneous identifications, the residues that could be identified are reported because they demonstrate the great degree of homology among the δ chain and the other

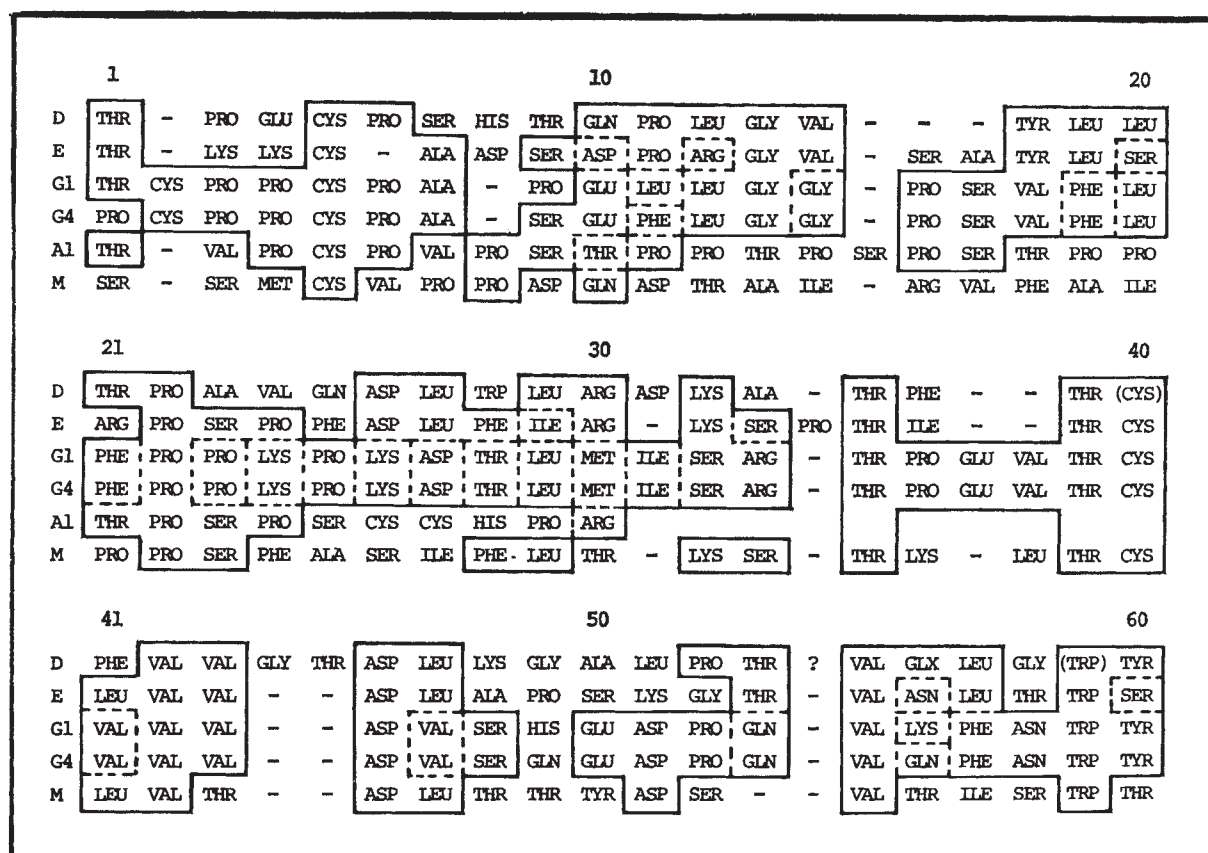


Fig. 2 Comparison of amino acid sequence of the IgD Fc fragment with the analogous sequences of other Ig heavy chains⁹⁻¹³. Gaps were inserted to achieve maximal homology among all six proteins that were compared.

heavy chains whether determined in sections at the beginning or end of the sequenced Fc portion.

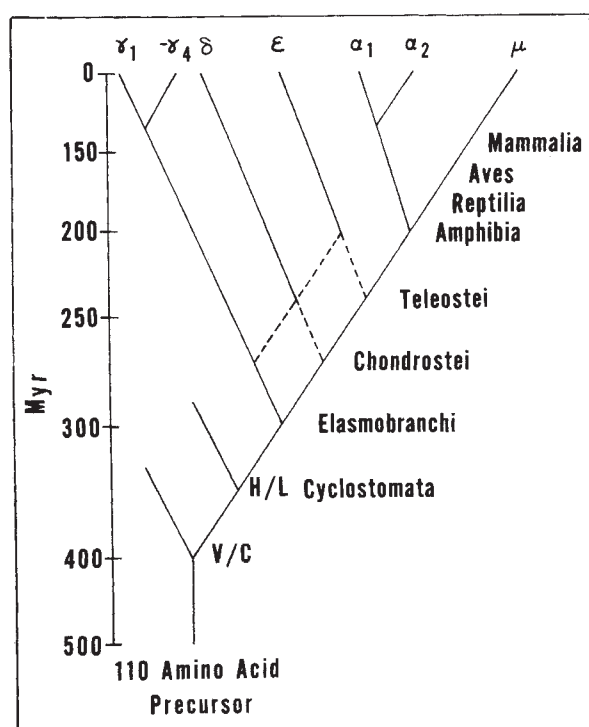
The δ chain and the other Ig heavy chains were strikingly homologous (Fig. 2) when the sequences at the beginning of the Fc fragment that contains the half-cystine residue involved in the inter-heavy-heavy chain disulphide bond were compared. Homologies with IgE (ref. 9) (41%) closely followed by IgG1 (ref. 10) and IgG4 (ref. 11) (34%) were greatest, whereas IgM (ref. 12) showed the least homology (23%). Relatively little is known about the amino acid sequence of IgA in this area; nevertheless, a hinge peptide of IgA1 (ref. 13) had a reasonable degree of homology (24%) and was probably also derived from the beginning of the Fc fragment. The degree of homology to any given Ig class was similar over the entire area of the Fc fragment that was sequenced. It is therefore most likely that the remaining portion of the δ chain's constant region will show a similar degree of homology to the other heavy chains.

Little information is thus far available on the COOH-terminal domain of the δ chain. COOH-terminal analyses suggested the sequence Pro-Gly-COOH (ref. 6) similar to that of IgG (Pro-Gly-COOH) and IgE (Pro-Gly-Lys-COOH) and contrasting to those of IgM and IgA (Cys-Tyr-COOH). Like the ϵ chain, the δ chain lacked a methionine residue near the COOH-terminus⁶ that was found in γ , α and μ chains. IgD has a high carbohydrate content⁶ resembling that of IgE, IgA and IgM and differing markedly from that of IgG.

Based on these data and resemblance of the different heavy chains to one another I have attempted to place the δ chain into an evolutionary tree that was previously proposed for the other Ig polypeptide chain genes^{14,15} (Fig. 3). The relatively large difference in structure between the δ chain and the other heavy chains suggests that it evolved rather early. Because γ and

μ chains differ the most, they probably diverged first, perhaps about 300 Myr ago¹⁵. The δ chain and ϵ chain probably

Fig. 3 Scheme of possible evolution of Ig polypeptide chain genes.



evolved next, either from a primitive γ - or μ -like heavy chain, 200-300 Myr ago (before the appearance of mammals). The time span of the appearance could be anywhere between 200 and 300 Myr ago, because it has been estimated that the α chain which shows greater homology to the μ chain evolved about 200 Myr ago. Obviously, phylogenetic studies of IgD will also be necessary to place evolutionary origin of IgD with greater certainty. If as predicted, however, IgD evolved before mammals appeared, one should be able to find an Ig class analogous to human IgD in lower vertebrates such as teleosts, amphibians, reptiles and birds.

Unfortunately, IgD is only a minor serum component¹, and it has been extremely difficult to isolate it from serum without a specific antiserum. Since IgD seems to be a lymphocyte receptor, however, an approach recently used to demonstrate an IgD analogue in mice^{16,17} could be applicable to the problem. These investigators first labelled the lymphocyte surface Ig and precipitated the IgM with a μ chain specific antiserum; in a second step they precipitated any other Ig that was present on the lymphocyte membrane with an anti-light chain antiserum. An Ig with characteristics resembling IgD was then discovered. Attempts to demonstrate this IgD-like substance on foetal murine lymphocytes were unsuccessful, but IgM was demonstrable by this method, suggesting that in ontogeny IgM appeared before IgD. Since ontogeny often parallels evolution, this finding could corroborate the position of IgD proposed in Fig. 3.

Although the function of IgD is still unknown, an early appearance and persistence in evolution would suggest its importance in the immune system. The striking resemblance of IgD to IgE and IgG, both known to have cytophilic properties¹⁸, led us to study the binding of IgD myeloma proteins to lymphocytes, neutrophils and monocytes; however, no binding to any of these cell types could be demonstrated¹⁹. It seems, therefore, most likely that IgD is a lymphocyte receptor the function of which may be quite different from that of IgM, to which it bears the least homology.

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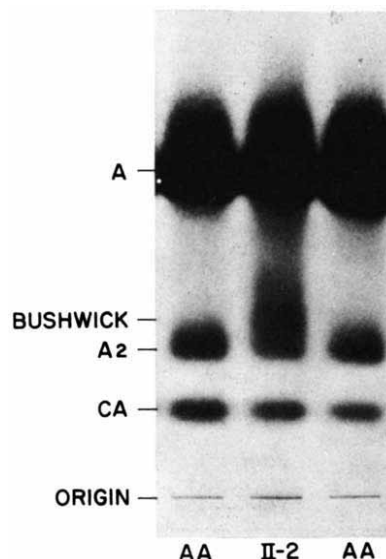
Rapid postsynthetic destruction of unstable haemoglobin Bushwick

MOST structural variants of human haemoglobin occur in smaller amounts than HbA in the peripheral blood of heterozygous subjects. Abnormal haemoglobins with mutations in the β chain usually comprise 35-45% of the haemoglobin in haemolysates. In contrast, haemoglobins with abnormal α chains generally amount to only 20-25% of the total haemoglobin. This difference in the proportion of α and β chain variants is thought to reflect the fact that in some populations there are two α loci and only one out of four α globin genes is affected in a heterozygote, whereas one out of two β globin alleles is abnormal in a subject with a β variant^{1,2}. Certain unstable haemoglobins with critical alterations in β -chain structure also constitute a much smaller proportion of the total circulating haemoglobin than HbA. The low concentration of these abnormal haemoglobins is the result of accelerated preferential destruction³⁻⁷. We report here a new unstable β variant, Hb Bushwick, which was detected in an intact form constituting only 1-2% of the total circulating haemoglobin. Evidence was found for rapid postsynthetic destruction of the abnormal β chain. In spite of this accelerated loss of the product of one of the two β alleles, the affected erythrocytes contained normal amounts of haemoglobin.

The variant haemoglobin was discovered during the investigation of an Italian-American woman with a mild chronic anaemia and a history of several episodes of moderately severe haemolysis and jaundice associated with drug administration and intercurrent infections. The presence of an unstable haemoglobin was suggested by the generation of multiple, small, coccoid, intraerythrocytic inclusion bodies on incubation of the subject's blood with the redox dye new methylene blue⁸. Starch gel electrophoresis in Tris-EDTA-borate buffer, pH 8.6 (ref. 9), revealed a faint abnormal haemoglobin band just anodic to HbA₂ (Fig. 1). Mild, compensated, chronic haemolysis, slightly increased reticulocyte levels (2.0-5.7%) and the abnormal haemoglobin were also demonstrated in five relatives of the proband (Table 1).

Hb Bushwick was purified by column chromatography on

Fig. 1 Starch gel electrophoresis in Tris-EDTA-borate buffer, pH 8.6, of haemolysates from (AA) normal individual and (II-2) proband with Hb Bushwick stained with Amido Black. The anode is towards the top.



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Table 1 Haematological values of family members

Subject	Hct (%)	Hb (g%)	RBC ($\times 10^6$ per μ l)	MCV (μ l)	MCH (pg)	MCHC (%)	Reticulocytes (%)	Abnormal electrophoresis	Inclusion bodies
Father (I-1)	40.5	13.1	4.23	96	31	32.4	3.6	+	+
Mother (I-2)	40.0	12.9	4.98	80	25.9	32.2	1.4	—	—
Propositus (II-2)	36.2(33.3)*	11.1(10.5)	3.76(3.42)	96(97)	30.1(31.6)	30.6(30.7)	4.1(15.0)	+	+
Sister (II-4)	36.0	12.2	3.91	92	31.0	34.8	3.3	+	—
Brother (II-7)	46.0	16.6	5.58	82	29.8	36.1	1.6	—	—
Son (III-1)	46.3	16.1	5.36	86	30.1	35.4	1.0	—	—
Son (III-2)	42.0	14.1	4.69	89	30.1	34.2	5.7	+	+
Nephew (III-4)	36.4	11.7	4.72	78	24.8	32	2.0	+	—
Nephew (III-5)	35.5	12.1	3.89	91	31.1	34.1	3.1	+	—

*Figures in parentheses represent values obtained in acute haemolytic episode.

DEAE-cellulose in Tris-phosphate buffer, pH 8.6 (ref. 10). When estimated by haem absorption at 540 nm, the proportion of Hb Bushwick in the haemolysate was 0.7–1.2% whereas the concentration of HbA₂ was 1.2–2.4%. When the relative haem and protein contents of haemoglobin fractions prepared by chromatography were compared, however, the ratios of the parameters reflecting haem content (pyridine haemochromogen¹¹ and absorbance at 420 and 540 nm) to the parameters of protein concentration (absorbance at 280 nm and total nitrogen content), were one-third to one-half lower in the Hb Bushwick samples than in the HbA samples. This suggests that Hb Bushwick is partially depleted of haem groups.

Table 2 Amino-acid analysis of β^{Bushwick} TpIX

	β^{A} TpIX	β^{Bushwick} TpIX	Difference
Val	1	1.7 (2)	+1
Leu	4	3.9 (4)	
Gly	2	1.1 (1)	—1
Ala	2	2.1 (2)	
Phe	1	1.1 (1)	
Ser	1	0.9 (1)	
Asx	3	2.9 (3)	
His	1	0.9 (1)	
Lys	1	1.0 (1)	

The α and β chains of Hb Bushwick were separated by column chromatography on CM-cellulose in 8 M urea at pH 6.7 (ref. 12). Both polypeptides eluted in the positions expected for the corresponding normal globin chains. The two globin fractions were amino-ethylated, digested with trypsin and peptide maps prepared¹². The fingerprints of both chains of Hb Bushwick were identical to the patterns obtained with

the corresponding polypeptides derived from HbA. All the peptides of both the α and β chains of Hb Bushwick were therefore subjected to amino-acid analysis. The composition of β^{Bushwick} TpIX (positions 67–82) indicated that there was one more valine residue and one less glycine residue than expected for β^{A} TpIX (Table 2). In β^{A} TpIX, glycine occupies positions 69 and 74. When β^{Bushwick} TpIX was digested with chymotrypsin the resulting peptide corresponding to positions 67–71 had a normal composition, whereas the chymotryptic peptide corresponding to positions 72–75 had the composition Ser, Asp, Val, Leu. This result indicated that the substitution of valine for glycine is at position 74 in the β chain of Hb Bushwick.

Glycine at β 74 occupies position 18 in the E helix and faces inward towards the haem crevice in a crowded cleft which separates the E helix, the EF corner, and the F helix¹³. Glycine is invariant in this position for all known β chains except the kangaroo¹⁴. Introduction of an amino acid with a larger side chain into this tightly packed area would be expected to make the molecule unstable. The insertion of the large valine residue into the scale model of haemoglobin at position β 74 brings the γ carbons of valine closer than the minimal permissible van der Waals distance to several atoms in serine β 70 (E14), threonine β 84 (EF8) and leucine β 82 (EF6) (M. F. Perutz, personal communication). These short contacts would force apart the E and F helices between which the haem group is suspended. This widening of the haem pocket could weaken several haem–globin contacts which probably accounts for the loss of haem from Hb Bushwick. Another human haemoglobin mutant, Hb Shepherd's Bush, has a substitution of aspartic acid for glycine at β 74 (ref. 15). The side chain of aspartic acid is thought to be able to swing out of the haem pocket towards the surface avoiding complete disruption of

Table 3 Incorporation of ³H-leucine in haemoglobin by reticulocytes

Time (min)	Haemoglobin	Total radioactivity* (c.p.m.)	Total radioactivity† (c.p.m.)		Specific activity c.p.m. per mg*†	
			α	β	α	β
15	A	91,800	4,647	17,521	58	198
	Bushwick	30,276	1,942	5,555	992	3,676
50	A	434,330	18,125	58,156	290	934
	Bushwick	111,720	6,781	8,165	6,576	8,760
5	A	82,464	13,150	17,128		
	Bushwick	33,191	2,795	6,916		
120	A	2,463,340	530,918	623,210		
	Bushwick	315,290	68,947	30,051		
5	A	65,219	25,476	39,743	140	243
	Bushwick	42,683	9,120	33,743	2,886	13,385
90	A	1,205,160	512,834	692,326	4,977	7,445
	Bushwick	294,252	196,168	98,084	73,660	45,280
5	Whole haemolysate		24,300	24,397		
90	Whole haemolysate		332,940	391,745		

*By DEAE cellulose chromatography.

†By CM cellulose chromatography.

‡Corrected for carrier protein.

the tertiary structure¹⁵. Hb Shepherds Bush, which comprises 24% of the haemoglobin in the blood of affected subjects, may thus be more stable than Hb Bushwick.

Because only an unusually small amount of Hb Bushwick could be demonstrated by haemoglobin electrophoresis or chromatography, attempts were made to detect the presence of additional amounts of β Bushwick in erythrocytes by amino-acid analysis. Since $\beta^{\text{A}}\text{TpIX}$ has a glycine-alanine ratio of 1.0 whereas that of $\beta^{\text{Bushwick}}\text{TpIX}$ is 0.5, βTpIX prepared from an unfractionated haemolysate would have an intermediate Gly-Ala ratio depending on the proportions of the two haemoglobins present. Analysis of peptide βTpIX of HbA purified from the haemolysate of the proposita revealed a Gly-Ala ratio of 1.0, suggesting that no Hb Bushwick was contaminating that fraction. Three analyses of peptide βTpIX obtained from whole globin prepared by direct acid-acetone treatment of the unfractionated haemolysate, including membranes, gave Gly-Ala ratios of 0.97, 0.93 and 0.92, indicating that the erythrocytes could contain as much as 5–15% of Hb Bushwick. Denaturation of the unstable haemoglobin or binding to cell membranes during preparation of a haemolysate could explain the lower amounts of Hb Bushwick demonstrable by chromatography or electrophoresis.

The relative synthetic rates of HbA and Hb Bushwick were directly examined *in vitro*. Washed erythrocytes from the propositus were incubated for 5–120 min with ³H-leucine. The cells were lysed and HbA and Hb Bushwick were separated by DEAE chromatography. The radioactivity in each haemoglobin fraction was measured (Table 3) and globin was prepared. The α and β chains were separated and the total radioactivity and specific activity of the fractions were determined (Table 3). After incubation for 5–15 min the total radioactivity incorporated into β^{A} was only 1.2–3 times that in β^{Bushwick} . This indicated that the two β globin chains were synthesised in grossly equivalent amounts and suggested that the low proportion of Hb Bushwick found in blood was a result of postsynthetic loss of the unstable variant. This rapid loss of newly synthesised Hb Bushwick was confirmed by the steep rise in the ratio of the radioactivity in β^{A} and β^{Bushwick} to 7–20:1 after 50–120 min incubation. A pool of non-radioactive excess free α chains in the erythrocytes was suggested by the low specific activity of the α chains relative to β chains in both haemoglobins after short periods of incubation. The excess α chains were a result of degradation of preformed Hb Bushwick and were not the result of a deficit in β globin synthesis. Synthesis of α and β globin was balanced in an experiment in which the entire haemolysate, including membranes, was converted directly to globin before chain separation (Table 3).

In spite of the evidence of rapid postsynthetic loss of Hb Bushwick, the erythrocytes did not seem hypochromic when examined microscopically and exhibited a normal mean corpuscular haemoglobin of 31 pg (Table 1). These findings suggest that the normal trans β^{A} allele was able to compensate for the loss of Hb Bushwick by increasing β^{A} globin synthesis. There are several other examples of subjects having unstable β chain variants in amounts less than 20% of the total circulating haemoglobin^{16–23}. In all reports, except one¹⁶, the erythrocyte mean corpuscular haemoglobin was normal, or nearly so, and the red cells appeared normochromic when examined under the microscope. Regardless of whether the low intracellular concentrations of these unstable haemoglobins was primarily a result of increased destruction or decreased synthesis, a compensatory increase in β^{A} synthesis directed by the trans allele had to occur. Such a degree of compensation is lacking in the β -thalassaemia trait⁹ where a defect in one of two β globin alleles is associated with a decreased cellular content of β globin mRNA (ref. 24), decreased HbA production and a low mean corpuscular haemoglobin content⁹. The trans β^{A} gene thus does not seem to be able to compensate by increasing its gene product in thalassaemic cells as effectively as in cells with the unstable haemoglobins. Possible mechanisms

for such failure of compensation in thalassaemia, include the presence of an inhibitor of β -globin synthesis or the lack of a synthesis-promoting substance. These mechanisms have been postulated previously^{25,26} but confirmation has been lacking. It would seem worth while to continue exploration in this area.

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High cooperativity of haemoglobin M Boston in the completely reduced state

HAEMOGLOBIN (Hb) M Boston and Hb M Iwate, in which distal and proximal histidines, respectively, in the haem pockets of α chains are replaced by tyrosines, bind ligands only to the normal β chains, and have α chains stabilised in the ferric form. These M Hbs contain mutant α chains and have abnormally low oxygen affinity, almost no Bohr effect and virtually no haem-haem interaction^{1,2}. The β chain mutants, Hb M Hyde Park and M Saskatoon, have normal oxygen affinity and a

normal Bohr effect but show almost no haem-haem interaction^{3,4}. Thus among the M Hbs, the proximal and distal mutants of the same chain display similar ligand binding properties. When abnormal α chains of Hb M Iwate are completely reduced using dithionite, the affinity for CO and Bohr effect were almost normal, but Hill's constant was still one⁴. It has been suggested that the ligand binding properties of completely reduced Hb M Boston are approximately the same as those of Hb M Iwate^{5,6}. We have studied the equilibria of Hb M Boston and Hb M Iwate in both the half ferric and completely reduced states using ethylisocyanide (EIC), a non-oxidising ligand which equilibrates rapidly with haemoglobin, and here report that the completely reduced Hb M Boston shows ligand binding properties similar to those of Hb A.

Hb M Boston and Hb M Iwate were separated from Hb A using an Amberlite CG-50 column (1.5 $\times$ 40 cm) previously equilibrated with 0.05 M phosphate buffer, pH 7.0. After Hb A had been washed out with 2 l of the same buffer, Hb M remaining on the column was eluted using a linear gradient from 0.05 to 0.2 M phosphate buffer (pH 7.0) with 300 ml of each buffer. Purity was examined by starch gel electrophoresis of methaemoglobin M, prepared by oxidation of the sample with potassium ferricyanide.

Completely reduced Hb M was prepared by bubbling with O gas (helium-isobutane, 99.05 : 0.95), subsequent addition of 3 mg ml⁻¹ sodium dithionite and standing overnight at room temperature in a cell with a light path of 1 cm and a rubber stopper seal. Half ferric Hb M Iwate, in which normal β chains contain ferrous haems and abnormal α chains ferric haems, was prepared by deoxygenation of the sample solution and the addition of sodium ascorbate to give a final concentration of 5 mM. Half ferric Hb M Boston was prepared as follows. Completely reduced Hb M Boston was separated from dithionite by passage through a Sephadex G-25 column, and abnormal α chains were oxidised using dissolved oxygen for 20 min at room temperature. Absorption spectra of half ferric Hb M Iwate and Hb M Boston showed that only abnormal α chains were ferric. Equilibrium with EIC was determined spectrophotometrically following the method of Anderson *et al.*⁷.

The EIC dissociation curves for Hb M Iwate at various pH are shown in Fig. 1. In the half-ferric state, CO affinity was very low and the Bohr effect very small. When abnormal α chains were reduced and all haems in Hb M Iwate were capable of binding EIC, affinity was a little greater than that of Hb A and

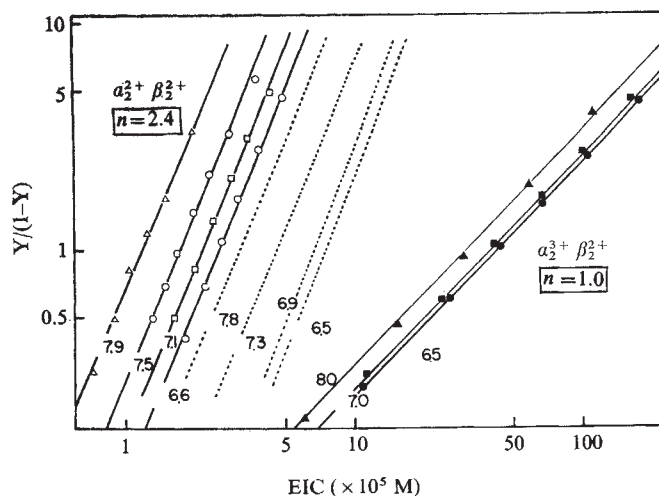


Fig. 2 EIC equilibrium curves for Hb M Boston. Experimental conditions and symbols used are as in Fig. 1.

the magnitude of Bohr effect was almost normal. Hill's constant, however, was approximately one in both the states. These results are qualitatively similar to those obtained for CO binding to Hb M Iwate⁴. EIC equilibrium curves for half ferric Hb M Boston were almost the same as those for Hb M Iwate (Fig. 2). Completely reduced Hb M Boston, however, showed haem-haem interaction as strong as Hb A, although its affinity and Bohr effect were similar to those of completely reduced Hb M Iwate.

X-ray analysis of Hb M Boston shows that the ferric iron atoms in the abnormal α chains are bonded to the phenolate side chains of the tyrosines which have replaced the distal histidines⁵. The α haem groups of Hb M Iwate are probably bonded to both the distal histidines and proximal tyrosines⁶. The resulting changes in tertiary structure of the α -chain mutants are considered to stabilise the haemoglobin tetramer in the quaternary T structure, which causes the abnormal ligand binding properties^{5,6}. When the iron atoms of the α haems of Hb M Boston are reduced by dithionite, bonds between the haem irons and the phenolates may be broken and bonds with the proximal histidines may be formed. The resulting structure seems to be similar to that of Hb A as judged from the EIC binding properties. Similarly, reduction of ferric irons in abnormal α chains of Hb M Iwate may break bonds between α haem irons and distal histidines, which increases both the ligand affinity and Bohr effect. Replacement of proximal histidine by tyrosine in Hb M Iwate, however, abolishes the cooperativity in EIC binding; the importance of the proximal histidine in cooperative ligand binding is suggested.

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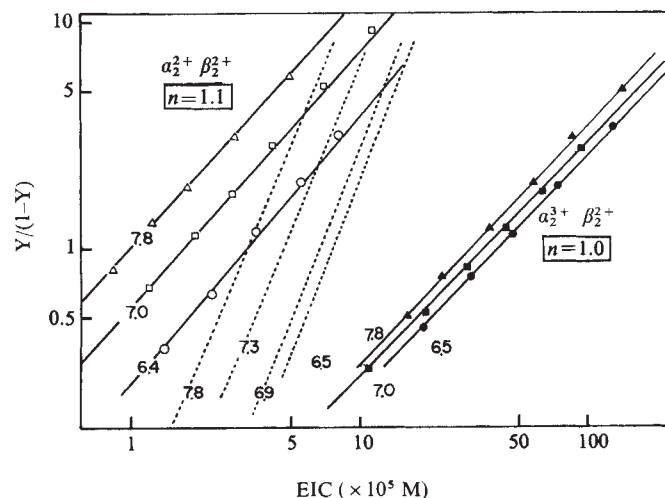
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Fig. 1 EIC equilibrium curves of Hb M Iwate in 0.2 M phosphate buffer at 25°C. The fractional saturation of haemoglobin with EIC, Y , against the EIC concentration, c , is plotted according to a modified form of Hill's equation, $\log Y/(1-Y) = n \log c + \log K$. Open and filled symbols represent the experimental points for the completely reduced and the half ferric state, respectively. —, Hb M; ---, Hb A. pHs shown beside plots.



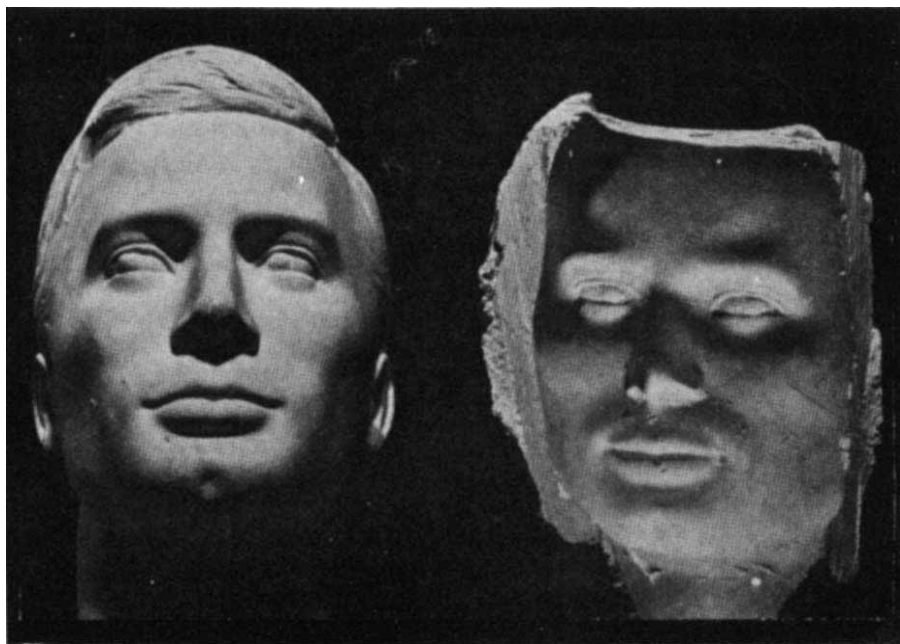
reviews

How man has seen the world

THE declared aim of this ambitious handbook* is "to bring together essential aspects of the very large, diverse and widely scattered literature on human perception." The present volumes are the first of 12; a third volume, *Biology of Perceptual Systems*, was published in 1973. Volumes 4-6 will cover the individual senses; and in the last six volumes it is planned to include areas such as speech and language, aesthetics, the perception of form, of space and of objects, and the perceptual aspects of thought.

Yet this is not a handbook. It is a very large collection of essays. There are many notable chapters by individual contributors, but the sum of the parts cannot fairly be represented as a true handbook. Cross-references within and between volumes are almost completely absent and there is much internal evidence to suggest that contributors have not read each other's chapters: thus, R. M. Boynton, who gives an account in Volume 1 of J. J. Gibson's theory of visual perception, cannot have been invited to read those passages in the same volume in which M. Wertheimer makes the elementary mistake of speaking of visual receptors firing impulses.

Indeed, there is grave doubt as to whether the editors themselves have read all the contributions. Very often the same ground is covered two or three times without comment or cross-reference, so that the reader is left wondering whether he is missing some subtlety; there seem, for example, to be three introductions to epistemology, two introductions to psychophysics and two accounts of multidimensional scaling. The very sequence of chapters is sometimes bizarre: a chapter by J. R.



Though both are viewed under the same lighting and have the same texture, the face on the right does not appear as a mould of that on the left.

Royce on epistemological psychology appears, inexplicably, in the historical section of Volume 1, whereas, if it belonged anywhere in that volume, it would have been as a bridge between the philosophical and the psychological sections. Similarly, in Volume 2, a relatively technical paper by A. Sandusky on memory effects in psychophysics is separated from A. Parducci's paper on contextual effects and is instead inserted between a short and very readable introduction to psychophysics by F. Nowell Jones and a long and very readable introduction to psychophysics by E. Galanter. In short, in spite of the 2 editors and 17 members of the editorial board, almost the only evidence of an editorial hand is the use throughout of *cf* where *v* is properly required.

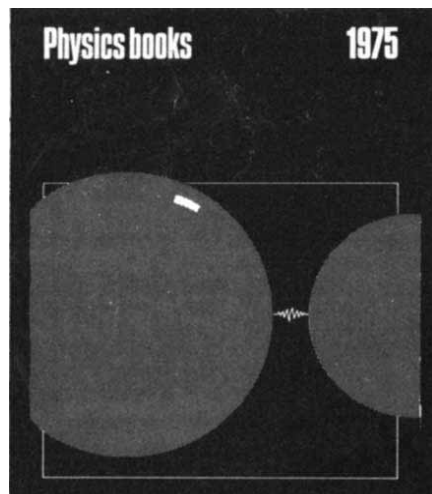
The editors must, however, enjoy some credit for their choice of contributors. Many of the individual chapters are of a very high standard, particularly Hochberg's excellent review of the history and present state of the Gestalt tradition; Berlyne's introduction to attention; Garner's introduction to attention; and the comprehensive account of multidimensional scaling by Carroll and Wish. Luce and Green combine to write a supplement to the 1966 book on signal detection theory by Green and Swets. R. L. Gregory, in a section of Volume 1 devoted to grand

theories of perception, does what the editors have failed to do and contrasts different theoretical approaches, considering the capacity of each to accommodate a number of facts that he has carefully chosen to support his own view that "perceptions are hypotheses". Elsewhere in the same volume there is a critique of this class of theory by Metzger but, characteristically, there are no cross-references between his chapter and those by Gregory and Hochberg.

Although Volume 1 is entitled *Historical and Philosophical Roots of Perception*, the history of perceptual theory is covered very inadequately. The origins of the sensory sciences in physics and medicine are almost completely neglected, as is the history of the study of senses other than vision. Such history as is dealt with reads soundly, but it is often drawn from secondary sources and professional historians of science would judge it presentist and Whiggish. In recent years historians of science have notoriously drawn upon psychological illustrations of the hypothetical nature of perception and thus it is the more unhappily ironic that this opportunity has not been taken to introduce a more hermeneutic approach to the history of perceptual theory and to discuss theories of perception in the context of the way man has seen the world.

J. D. Mollon

*Handbook of Perception. Vol. 1: *Historical and Philosophical Roots of Perception*. Pp. xix+431. \$23.50; £11.30. Vol. 2: *Psychological Judgment and Measurement*. Pp. xix+556. 9.50; £14.15. Edited by Edward C. Tretter and Morton P. Friedman. Academic: New York and London, 74.)



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Model standard for hydrogen bonding

Hydrogen Bonding. By Melvin D. Joesten and L. J. Schaad. Pp. vi+622. (Marcel Dekker: New York, 1974.) \$45.00.

THIS book is explicitly planned as supplemental to Pimentel and McClellan's *Hydrogen Bond*, "... to provide comprehensive coverage of those topics which account for a large percentage of the hydrogen bonding (HB) papers published since ... (1960)". It reaches a standard not unworthy of its model.

Four of the five excellent chapters are by Professor Joesten, and cover the detection of HB, thermodynamics and kinetics, (spectroscopic) correlations, and intramolecular and homo-intermolecular HB. The other chapter, by Professor Schaad, deals with the theory of HB exceedingly well; with minimal mathematics, he surveys the application of quantum mechanics to molecules, carefully explains the types of approximation necessary with HB, and assesses their limitations—all this with masterly elegance. He ends his chapter with a list of theoretical studies of HB systems carried out between 1960 and 1973. He

writes: "It is tempting for the experimentalist to conclude that since few theoretical predictions are completely certain one can ignore all theoretical results. The difficult alternative of weighing each separately is probably more useful".

Besides some 250 pages of straight text, there are 130 pages of tabulated results and 2,703 references. The book is largely concerned with spectroscopic studies and their thermodynamic consequences. This is the way most of the current HB literature runs; it implies no adverse criticism to state that this book concentrates on relatively weak HB; that it has less to say about strong HB or its study by diffraction methods. The book is an example of the pleasing results that are sometimes achieved by photo-offset. There are few errors—something is awry with the legend to Fig. 5.1. The price will deter most individuals from buying their own copy; but perhaps these days even 4 pence a page is not preposterous for so valuable a book.

J. C. Speakman

Limited horizons

Television review by Robbie Vickers

THE BBC are to be commended in launching *Choices for Tomorrow*, a new series of half-hour programmes on the environment and how we use or misuse it. The first, *Woodland or Wasteland* (Monday April 7; 11.05 p.m.) went only part of the way to solving the problems facing educational television.

Michael Pye of the *Sunday Times*, aired his own personal views on the trials and tribulations of the tree in Britain—government forestry policy, recycling of paper products, and so on. His impressions—they were essentially his, and on a topic very much related to his own sphere of interest—were interesting and should provoke thought and discussion.

The comments on the way we are likely to affect the world we live in were, however, left too open-ended, making the whole programme look very much like a miniature and limited *Horizon*. In the time available, a 'chat' programme of this sort cannot attempt to supply definitive answers—and we wouldn't want this—but it could forward suggestions; it is simply not constructive never explicitly to state alternatives. Such comments as "every time I write an article, the paper publishes one and a quarter million copies of it and that means 4,000 trees die"—do not get us anywhere other than making us aware of the problems.

Before making suggestions, however,

perhaps we should get our facts straight. At the moment, the cost of de-inking and bleaching paper far outweighs the benefits that may accrue from its re-use. It is not until a balance is reached that this method of conserving a resource can become viable and economical, although the organised collection of waste paper is a method worth investigating on a commercial scale.

For some time now, the Forestry Commission have, as a matter of high priority, considered the integration of maximum wood production and the amenity value or aesthetic value of our woodlands. National Parks were conceived to meet this need, at a time when the idea of increased leisure time came to the fore. To some people, therefore, Mr Pye's ideas may have been misleading, particularly at such a late hour in the evening.

What was given was a popularised, one-sided, slightly inaccurate view. At least there was sufficient comedy and wit to sustain enough interest for people to take the punch-lines, but people need easily accessible informed comment to make reasoned judgements about the world in which they live.

Allowing for the limitations and the personal nature of the views expressed, with a bigger budget, more time and a research staff, this programme could become worth a slot during peak viewing hours. Future programmes will consider the energy shortage, pollution control and the effects buildings have on our behaviour.